Effects of Plant Protection Compounds on Wheat Gene Expression

Dissertation

zur

Erlangung der naturwissenschaftlichen Doktorwürde

(Dr. sc. nat.)

vorgelegt der

Mathematisch-naturwissenschaftlichen Fakultät

der Universität Zürich

von

Frédérique Pasquer

aus

Frankreich

Promotionskomitee:

Prof. Dr. Beat Keller
Dr. Catherine Feuillet
Prof. Dr. Robert Dudler

Zürich 2005

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Summary

Wheat is cultivated world wide on large areas and agrochemical treatments are used in order to limit yield losses from insect and pathogen attacks and from competition of weed for nutrients and light. The mode of action of these chemical compounds on their biological target is usually well known, but their effect on plant metabolism is less well studied. Pesticides are efficient for protection of crops, but these molecules can potentially have an effect on plant metabolism which might change the nutritional quality of the product.

In this thesis, gene expression of plants treated with several commonly used pesticides was compared to untreated plants grown under the same conditions, in order to determine if agrochemicals have an impact on wheat metabolism. These effects were assessed under two different growth conditions: a controlled environment (greenhouse or phytotron) and in the field. A microarray containing 600 cDNAs from barley was established during this thesis to determine transcript levels in wheat. Cross-hybridisation of barley and wheat transcripts is commonly observed because these species show high sequence homology. Several parameters were tested to find the best materials adapted to our cDNA spotting device. Microarray chips allowing good hybridisation results were finally obtained when poly-L-lysine coated slides were spotted with DNA resuspended in a buffer containing DMSO.

The impacts of two fungicides, azoxystrobin and fenpropimorph, and of the systemic acquired resistance (SAR) enhancer, BTH, on wheat gene expression were specifically visible in the greenhouse growth conditions. Defence-related genes showed high expression levels after treatment with all these three compounds, with BTH being the strongest inducer of this set of genes. Furthermore, these genes were still up-regulated two weeks after the treatments albeit at a lower level than after 24 hrs. Azoxystrobin treatment resulted in a weaker induction of the defence-related genes for all time points. Furthermore, the WCI2, WCI1 and WCI4 genes that showed the highest over-expression level after the two other treatments were not induced 24

hrs after azoxystrobin treatment, reflecting a specific response triggered by this fungicide. In the field, no gene showed any differential expression after the treatments. This result can originate from the constitutive high level of expression of the defence-related genes in this environment and can also be explained by the fact that plants have different responses when submitted to consecutive stresses.

Further analyses of the control plants in the field and in the greenhouse using the Barley1 GeneChip from Affymetrix confirmed differences of expression in the two environments for untreated plants. Defence-related genes and genes encoding proteins involved in protein maturation were expressed at a higher level in the field as compared to the greenhouse. In particular, a putative lipid transfer protein gene was 90-fold up-regulated in the field. This gene belongs to a large family, classified as PR14, containing members involved in defence signalling. Further characterisation of this gene should demonstrate if it is involved in this pathway.

The treatment of wheat with herbicides resulted in specific differences in gene expression depending on the compound used (2,4-D, cinidon-ethyl and tribenuron-methyl). Under controlled conditions, the auxin-like herbicide 2,4-D triggered the expression of genes from the phenylpropanoid pathway after 24 hrs only. Cinidon-ethyl treatment strongly induced peroxidase genes as well as defence-related genes after 24 hrs and after one week. In contrast, the effect of tribenuron-methyl was only visible after one week by the induction of defence-related genes. In the field, no differential expression was observed early after 2,4-D treatment but defence-related genes were down-regulated after one week. After cinidon-ethyl treatment in the field, the expression profiles were similar to the results obtained in controlled conditions, although lower induction ratios were observed. Interestingly, tribenuron-methyl induced the over-expression of the isopropylmalate synthase gene, involved in branched-chain amino acid synthesis, and defence-related genes after 24 hrs and 72 hrs. The up-regulation of

a gene from the biosynthesis pathway inhibited by the herbicide could be due to a less efficient detoxification mechanism in the field compared to the controlled environment. On the other hand, increase of defence-related gene transcripts could reflect a SAR-like secondary effect induced by cinidon-ethyl and tribenuron-methyl metabolites, albeit at a lower level and transiently if compared to the effect of BTH.

In conclusion, we have demonstrated here that agrochemicals have an effect on wheat gene expression although wheat is not the target organism of these compounds (with the exception of BTH). No gene from primary metabolism was affected by the different treatments, but the induction of defence-related genes was a common theme. If these up-regulated transcripts are translated into proteins, this gene induction can benefit the plant by increasing its defence potential. However, defence-related proteins have also been determined as allergens for humans and animals. The assessment of substantial equivalence, which is compulsory for genetically modified organisms, could be applied to treated plants as another criterion for food safety determination before commercialisation of new agrochemicals.

Zusammenfassung

Weizen wird grossflächig auf der ganzen Welt angebaut. Der Einsatz von Pflanzenbehandlungsmitteln (PBM) limitiert Ertragsverluste, welche durch Insekten- und Pathogenbefall sowie durch Konkurrenz von Unkräutern um Nahrung und Licht verursacht werden. Die Wirkungsweise von PBM gegenüber Schädlingen ist meistens gut bekannt, ihr Effekt auf den Metabolismus der Pflanzen hingegen wird oft nicht untersucht. PBMs schützen Getreide effizient, aber ein Effekt dieser Moleküle auf den Pflanzenmetabolismus, der möglicherweise eine Veränderung der Nährstoffqualität des Produktes bewirken könnte, kann nicht ausgeschlossen werden.

In dieser These wurde die Genexpression von Pflanzen - behandelt mit verschiedenen herkömmlichen Pestiziden - mit jener von unbehandelten Pflanzen unter den gleichen Bedingungen verglichen. Damit sollte herausgefunden werden, ob PBM einen Einfluss auf den Weizenmetabolismus haben. Diese Effekte wurden unter zwei verschiedenen Wachstumsbedingungen studiert, in einer kontrollierte Umgebung (Gewächshaus oder Phytotron) und im Feld. Ein Microarray mit 600 cDNAs aus Gerste wurde während dieser Arbeit hergestellt, um die Transkriptionsniveaus von Weizenproben zu bestimmen. Da Weizen und Gerste hohe Sequenzhomologie zeigen, wird zwischen diesen beiden Gräsern oft eine Kreuz-Hybridisierung beobachtet. Mehrere Parameter wurden getestet, um die besten Materialien, angepasst an unseren Microarray-Spotter, zu finden. Die besten Hybridisierungsresultate wurden erhalten, wenn Glasobjektträger mit poly-L-Lysin beschichtet wurden und DNA aufgetragen wurde, welche in einem DMSO-haltigen Puffer gelöst war.

Unter Gewächshausbedingungen war der Einfluss zweier Fungizide, Azoxystrobin und Fenpropimorph und eines Enhancers der systemisch induzierten Resistenz (SAR), BTH, auf die Genexpression in Weizen spezifisch sichtbar. Abwehrverwandte Gene zeigten nach der

Behandlung mit diesen drei Substanzen eine erhöte Expression, wobei die Induktion von Genen dieser Genklasse durch BTH am stärksten war. Des weiteren waren diese Gene auch zwei Wochen nach der Behandlung noch überreguliert, wenn auch auf einem tieferen Niveau als nach 24 Stunden. Azoxystrobinbehandlung resultierte zu allen Zeitpunkten in einer moderateren Induktion der abwehrverwandten Gene. Die WCI2, WCI 1 und WCI 4 Gene, welche die stärkste Induktion nach den anderen beiden Behandlungen zeigten, waren 24 Stunden nach der Azoxystrobinbehandlung nicht induziert. Dies lässt vermuten, dass von diesem Fungizid eine spezifische Antwort ausgelöst wird. Im Feldversuch zeigte kein Gen nach diesen Behandlungen eine veränderte Expression. Dieses Resultat kann von der konstitutiv hohen Expression der abwehrverwandten Gene in der natürlichen Umwelt herrühren, welche durch aufeinander folgende Stresssituationen im Feld ausgelöst wird.

Weitere Analysen von unbehandelten Kontrollpflanzen, durchgeführt mit dem Barley1 GeneChip von Affymetrix, bestätigten Unterschiede in der Genexpression in den zwei Umgebungen. Abwehrverwandte Gene und Gene, welche Proteine codieren, die in die Proteinreifung involviert sind, waren auf dem Feld stärker exprimiert als im Gewächshaus. Im Speziellen war ein mögliches Lipidtransferprotein im Feld 90-fach überexprimiert. Dieses Gen gehört zu einer grossen Familie, klassifiziert als PR14, wovon einige Mitglieder in die Signalübermittlung von Abwehrreaktionen involviert sind. Eine weitere Charakterisierung dieses Genes wird zeigen, ob es an diesem Signaltransduktionsweg beteiligt ist.

Die Behandlung von resistentem Weizen mit Herbiziden resultierte in differentieller Genexpression abhängig von der verwendeten Substanz (2,4-D, Cinidon-ethyl und Tribenuron-methyl). Unter kontrollierten Bedingungen löste das auxinähnliche Herbizid 2,4-D die Expression von Genen des Phenylpropanoid-Signaltransduktionswegs nur 24 Stunden nach der Behandlung aus. Cinidon-ethyl-Behandlung führte zu einer starken Induktion von Peroxidase- und abwehrverwandten Genen nach 24 Stunden und nach einer Woche. Im

Gegensatz dazu war der Effekt von Tribenuron-methyl nur nach einer Woche in der Induktion abwehrverwandter Gene sichtbar. Im Feld konnte früh nach der 2,4-D Behandlung keine Expressionsunterschiede festgestellt werden, aber abwehrverwandte Gene waren nach einer Woche herunterreguliert. Nach der Cinidon-ethyl-Behandlung im Feld konnten ähnliche Expressionsprofile wie unter kontrollierten Bedingungen beobachtet werden, wenn auch in kleineren Verhältnissen. Interessanterweise induzierte Tribenuron-methyl die Überexpression des Isopropylmalat-Synthasegenes, welches an der Synthese verzweigter Aminosäuren beteiligt ist. Weiter waren abwehrverwandte Gene nach 24 und 72 Stunden ebenfalls überexprimiert. Verglichen zur kontrollierten Umgebung könnte diese Hochregulierung eines Genes, welches an inhibitierten Biosyntheseweg beteiligt ist, durch einen ineffizienteren Detoxifizierungsmechanismus im Feld verusacht sein. Auf der anderen Seite könnte der Anstieg von Transkripten der abwehrverwandten Gene auf einen SAR-ähnlichen sekundären Effekt hindeuten, welcher durch Cinidon-ethyl und Tribenuron-methyl induziert wird, wenn auch auf einem niedrigeren Niveau und nur transient, im Gegensatz zum BTH-Effekt.

Zusammenfassend konnten wir zeigen, dass Pflanzenbehandlungsmittel einen Effekt auf die Genexpression in Weizen haben. Auch wenn durch die verschiedenen Behandlungen keine Gene des primären Metabolismus betroffen waren, so ist die Induktion von abwehrverwandten Genen dennoch häufig. Falls die überexprimierten Transkripte in Proteine translatiert werden, kann die betroffene Pflanze von einem erhöhten Abwehrpotential profitierten. Jedoch sind abwehrverwandte Gene auch als Allergene für Menschen und Tiere bekannt. Die Einschätzung der substanziellen Äquivalenz, die bei gentechnisch modifizierten Organismen obligatorisch ist, könnte auch bei behandelten Pflanzen als ein weiteres Kriterium für die Lebensmittelsicherheit herangezogen werden, bevor neue Pflanzenbehandlungsmittel kommerzialisiert werden.

I. General introduction

1.1 Wheat: an agroeconomic story

Since the 1950's, the world population has rapidly increased and several major developments have dramatically changed agriculture in order to provide enough food (Evans, 1998). Crop yield has been improved by selection of high-yielding varieties. In addition, the use of fertilisers has allowed the use of land that was lacking necessary nutrients like nitrogen and phosphate. Finally, control of pests by agrochemicals has helped to reduce the yield losses.

Cereal production has strongly benefited from the agricultural improvements with a doubling of yield since the beginning of the 20th century (Evans, 1998) and cereals are one of the major food sources worldwide (WHO/GEMS/Food, 2003). Rice, maize and wheat still provide 11% to 41% of the daily food supply in Europe as well as in Asia (Table 1). Rice consumption is higher in Asian and African countries, whereas wheat remains the most important cereal in Middle Eastern and European countries. This geographical separation can be explained by the origin of domestication of these species (in the Fertile Crescent for wheat and from North India to South-East China for rice (Harlan, 1992)) and a better adaptation of the species to the climate of these regions. Cultivation of wheat covers nearly 20% of the cultivated area in the world, demonstrating its agricultural and economical importance. However, yield can be severely reduced by weeds which compete with wheat for nutrients and light, or by numerous insect pests and pathogens that attack wheat plants and seeds during storage. The management of weeds, diseases and pests with agrochemicals has become an important economic factor for farmers.

Table 1: Food regional diets in grams per person per day. Data from the Food Safety Department World Health Organisation 2003.

	MIDDLE EAST	**FAR EAST**	**AFRICA**	**LATIN AMERICA**	**EUROPE**[a]
Total Cereals	**429.9 (32.5%)**	**450.6 (41.6%)**	**291.7 (28.6%)**	**254.4 (18.7%)**	**221.9 (11.7%)**
Wheat	327.3 (24.4%)	114.8 (10.5%)	28.3 (2.2%)	116.8 (8.6%)	178.0 (9.3%)
Rice	48.8 (3.5%)	279.3 (25.7%)	103.4 (10.5%)	86.5 (6.4%)	11.8 (0.6%)
Barley	1.0	3.5	1.8	6.5	19.8
Maize	48.3	31.2	106.2	41.8	8.8
Total Root And Tubers	61.8	108.5	321.3	159.3	242.0
Total Pulses	21.2	14.5	17.6	20.6	9.4
Total Sugars And Honey	95.8	50.5	42.7	104.3	107.3
Total Nuts And Oilseeds	12.8	50.0	34.2	57.5	29.9
Total Vegetable Oils And Fats	40.7	14.2	23.2	21.9	38.8
Total Stimulants	8.2	1.7	0.6	5.5	14.4
Total Spices	2.6	3.1	1.8	0.5	0.5
Total Vegetables	233.0	178.9	77.0	150.4	371.6
Total Fish And Seafood	13.2	31.5	36.5	46.7	46.8
Total Eggs	14.6	13.1	3.7	11.9	37.6
Total Fruits	204.4	85.4	94.7	271.3	212.4
Total Milk And Milk Products	132.4	32.8	42.2	167.9	336.1
Total Meat And Offals	71.3	47.0	30.4	78.0	217.3
Total Animal Oils And Fats	0.9	1.8	0.6	5.4	10.6
TOTAL DIET IN GRAMS PER PERSON PER DAY	**1342.5**	**1083.5**	**1018.1**	**1355.5**	**1896.4**

[a]: Countries with European diet including Australia, Canada and the USA.

1.2 Pesticides

Perfect climate conditions for wheat production are rare and farmers have to react to adverse situations. Weeds and pathogens can severely affect crop yield but nowadays they can be controlled to some degree by agrochemicals, and they remain below an "economic damage level" where the costs to produce the crop are still lower than the returns of selling it (Benedict, 2003).

1.2.1 Recent rules

Since the last century, chemical control of weeds and pathogens has been widely used in agricultural production. However, recent evidence of the toxicity of some molecules, such as the insecticide DDT or the fungicide binapacryl, for the environment, ground water, wild fauna and flora as well as human health has led to a decreased use of agrochemicals (Duke, 1996). The Rotterdam Convention, adopted in 1998, and the International Code of Conduct on the Distribution and Use of Pesticides, adopted in 2002, have imposed new rules on hazardous chemical release and international trade in order to protect health and environment. Most of the commonly used pesticides have now to be submitted to different toxicological tests before commercial production, a long and costly process that has reduced the industrial research on new products as the number of different molecules with different targets is already large and sufficient to fight most of the pests (Duke, 1996).

1.2.2 Pesticide classes

The three major classes of pesticides are herbicides, insecticides and fungicides targeting weeds, insects and pathogenic fungi, respectively. The chemical industry has discovered and produced numerous types of very active compounds over the last decades.

1.2.2.1 Herbicides

Weeds can reduce crop yield by competing for nutrients and light. To prevent yield losses, the removal of these plants can be done either mechanically or chemically. The use of herbicides has dramatically reduced the amount of labour needed for pest control of the cultures and has also lowered the soil erosion due to cultural techniques (deep tillage) (Duke, 1996).

Herbicides are the most used pesticides worldwide. They are more or less selective and can either eliminate all type of plants or be very specific by targeting only some species (Sherman et al., 1996). They mostly act on biochemical pathways specific to plants. For example, the inhibition of biosynthesis of aromatic amino acids, which are essential for animals (i.e. not synthesised by them), can be achieved by glyphosate. Pathways specifically occurring in the chloroplast (photosynthesis) are targets of choice for herbicides as only plants should be damaged by these molecules (Tranel, 2003).

The resistance of weeds against various herbicides has arisen after repeated treatment with the same product in an area (Hall et al., 1994). A high selection pressure is created for weeds and this favours the development of mutant plants resistant to the product. Furthermore, the regular use of dicot-controlling herbicides will benefit monocot weeds, then inducing a “weed species shift” (Tranel, 2003). Therefore, the use of herbicides has to follow integrative pest management (IPM) approaches in order to avoid the development of weed resistance and weed species shift.

1.2.2.2 Insecticides

Insects feed on plants and can totally consume crops and all plants of a vast area (e.g. grasshopper invasion). The damage caused by insects amounts to about 20% of worldwide yield losses and one way to control such pest is the use of agrochemicals. Furthermore, the

control of insects is the only way to indirectly fight some plant diseases which are insect-transmitted, such as viral diseases.

Insecticides are mostly active on the neural system of insects by inhibition of the acetylcholinesterase (carbamates) or blocking of GABA receptors (phenylpyrazole (Zhao et al., 2003)). However, the toxicity of such molecules for animals and beneficial insects (honeybees) can be relatively high, depending on the molecular structure of the xenobiotic. Insecticides can also act on biosynthesis of chitin, the major constituent of insect exoskeleton. Chitin does not occur in vertebrates and compounds acting on this pathway should not have any impact on animal metabolism. Synthetic pheromones are also used to disturb interactions between insects leading to the disruption of insect reproduction (Reddy and Guerrero, 2004).

However, due to the high mobility of insects and high reproduction rates, resistance against the insecticides has frequently occurred. For example, in highly cultivated areas, the diamondback moth, *Plutella xylostella*, has developed multiple resistances against several insecticides (Sayyed et al., 2004). Resistance can originate from different mechanisms: improvement of detoxification, decrease of sensitivity at the site of action or low up-take of the xenobiotics (Plapp, 1976). Furthermore, the high structural similarities between insecticidal molecules can enhance the development of cross-resistances.

1.2.2.3 Fungicides

Fungi have a double impact on crops: first they can reduce yield during plant growth, secondly, they damage the harvest by the production of mycotoxins, which can be a risk for animal and human health (Bent, 2003). As breeding for resistant varieties is time-consuming due to difficulties to discover the genes responsible for resistance, fungicides remain the most efficient method to control fungal pathogens since the accidental discovery of the Bordeaux mixture in 1850’s (Knight et al., 1997).

Bordeaux mixture is active probably through its sulphur content which has recently been described as a phytoalexin, but its exact action on fungi remains elusive (Williams and Cooper, 2004). Chitin metabolism is also a target for fungicides such as polyoxins (Endo and Misato, 1969). Other modes of action of fungicides are based on the inhibition of mitochondrial respiration (strobilurins), of ergosterol biosynthesis (morpholins) or of methionine biosynthesis (anilinopyrimidines) (Knight et al., 1997). A product activating a fungal signal transduction pathway involved in osmotic stress response has recently been discovered (Kojima et al., 2004). A new way to fight fungal diseases is based on the discovery of compounds that can enhance the systemic acquired resistance (SAR) of the plant by inducing its defence mechanism before any pathogen attack occurs (Görlach et al., 1996). These products are preventively sprayed and can not only enhance the protection against fungal pathogens but also against bacteria and viruses (Knight et al., 1997).

Several fungal species have developed resistance against fungicidal compounds from all families. For example, the cereal disease *Magnaporthe grisea* can rapidly develop resistance when grown on media containing azoxystrobin (Avila-Adame and Koller, 2003). Therefore, research for molecules with new targets and new agricultural practices (IPM) with more rotation of compounds are developed and applied in order to delay resistance development.

1.3 Microarrays

The effects of chemicals on plant metabolism can be assessed by measuring gene expression, protein level or metabolite amount. We have chosen transcriptomic studies to determine the gene expression profile of wheat. Since the first study using a microarray chip containing 45 Arabidopsis genes (Schena et al., 1995), the microarray technique has been developed for many organisms and has allowed genome-wide expression profiling studies in some of them.

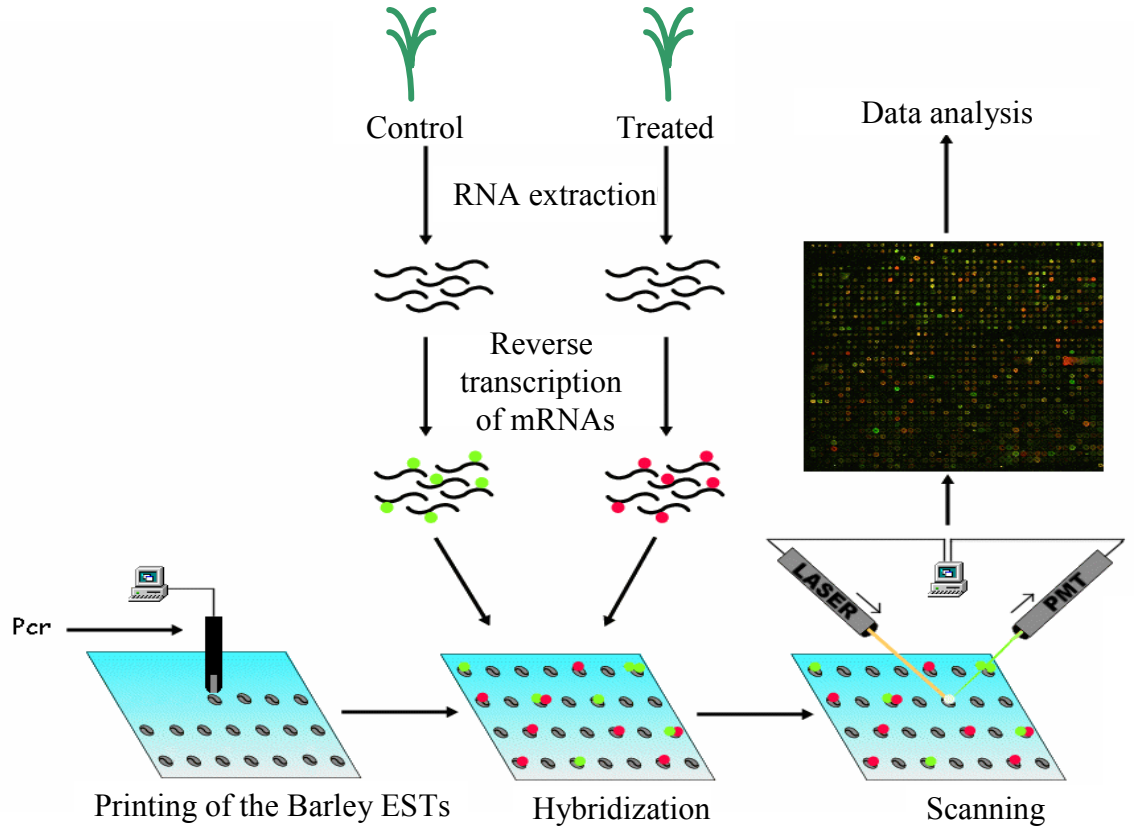


Figure 1: Microarray principle. First, cDNAs are printed on microarray slide. After RNA extraction of samples subjected to different treatments or growth conditions, mRNA from each sample is labelled with specific dyes (Cyanine-3 in green or -5 in red). Labelled nucleic acids are allowed to hybridise to the microarray, and slides are scanned at the dye-specific wavelengths. Finally, hybridisation intensities are determined by analyses of the resulting pictures with the Imagene software, Biodiscoveries Inc. (adapted from P. Marc)

1.3.1 The technical principle

A microarray chip is made of a solid support (usually glass slides coated with different substrates) that fixes, by covalent binding, DNA probes complementary to mRNA sequences. The labelled targets (cDNA or cRNA) derived from the studied mRNA samples are allowed to hybridize on the chip. The amount of mRNA originally present in the sample is then determined by the hybridisation intensity level, obtained by the scanning of the chip and the analysis of the resulting picture. cDNA microarrays allow the hybridisation of two samples that are differently labelled, usually with Cyanine-3 and -5 fluorochromes. The different steps of the technique are shown in Figure 1. Oligonucleotide microarrays, developed by Affymetrix (Lipschutz, 1999), allow the hybridisation of one sample only because of a different labelling technique, described in chapter IV.

Expression of thousands of genes can be studied simultaneously, in a relatively short time compared to usual Northern blot analysis. The microarray technique allows detection of genes with similar or different patterns of gene expression depending on the conditions studied. The result of such RNA studies was called transcriptome analysis. Transcriptomes reflect a precise biological state corresponding to the moment when the samples were collected (and directly frozen to prevent RNA degradation). The microarray results are thus more a snapshot and do not reflect a "general" expression pattern. Nevertheless, a lot of information can be obtained from such experiments and resulting expression patterns, for example by comparison between treated and untreated plants or between two lines only differing by a single mutation.

1.3.2 Sequence availability

The building of cDNA microarray chips for model organisms was facilitated by the sequencing projects for these species (human, mouse, yeast). Plant microarray studies were delayed due to the lower research funding for plants, the large number of interesting species, and the complexity of genomes. The Arabidopsis consortium has allowed the creation of

chips containing probes first covering 8,300 genes and then covering the total genome after the sequencing of Arabidopsis was completed in December 2000. Apart from the first paper on microarrays, studies on plant transcriptome analyses were published in 2000 for Arabidopsis and rice (Reymond et al., 2000; Yazaki et al., 2000) and 2002 for barley (Negishi et al., 2002).

The International Triticeae EST Cooperative (ITEC, http://wheat.pw.usda.gov/genome) has made several cDNA libraries publicly available since July 2000 (Matthews et al., 2003). The availability of this information had allowed to create a cereal-based microarray chip. Nearly one million Triticeae ESTs, more than half of which are from wheat, are now publicly available (data from December 2004). At the start of this thesis project, barley sequences were more numerous and the high similarity of coding sequences between cereals species and mostly between wheat and barley sequences (over 90%) allowed the use of a barley-based chip for wheat gene expression studies (Bennetzen et al., 1998; Feuillet and Keller, 2002; SanMiguel et al., 2002; Chantret et al., 2004). Furthermore, in 2003, Affymetrix has released a GeneChip based on barley cDNA sequences that contains more than twenty thousand probes that allow broad investigations on gene expression and cross-hybridisation with wheat samples has been demonstrated successfully (Close et al., 2004). Finally, in January 2005, an Affymetrix GeneChip representing more than fifty-five thousand transcripts of wheat was released.

1.4 Aim of the study

The effect of the chemical compounds used in wheat agriculture is well studied in their target species (although the mode of action of some products remains unclear) and at the toxicological level, but their impact on wheat itself is not well known. Genetically modified organisms are tested for substantial equivalence. In contrast, plants treated with xenobiotics which possibly modify their metabolism are not studied for substantial equivalence. The aim

of this thesis project was to investigate the gene expression profile of wheat after treatment with fungicides and herbicides. These transcriptome analyses were performed using a cereal microarray chip containing 600 cDNAs from barley, encoding key-proteins involved in primary and secondary metabolisms. The general behaviour of wheat in response to chemical treatments was analysed after growth either under controlled conditions (greenhouse) or under field conditions. Furthermore, the gene expression profiles of plants grown in these two conditions were further analysed using the Barley1 GeneChip from Affymetrix.

II. Establishment of the cDNA microarray technique

2.1 Setting-up of the cDNA microarray technique

The spotted DNA is described here as “probe” and the labelled cDNA as “target” following the terminology of Kane et al. (2000).

2.1.1 Amplification-purification of the clones

The aim of our project was to study gene expression in wheat. Barley and wheat are closely related species sharing high sequence homology in coding regions. At the start of the project, there were more sequences available from barley than from wheat. Therefore, a chip containing 600 barley clones was made.

To print barley DNA sequences only, without sequences of plasmid DNA backbone, an amplification step of the EST sequences was made. The ESTs from Clemson University were cloned in pBluescript SK(-) vector and amplified by PCR using the universal primers M13 (5’-GTAAAACGACGGCCAGT-3’ and 5’-GAAACAGCTATGACCATG-3’). Clones from the collection made in our laboratory were included in a different vector (pTriplEx2, Clontech) and Triplex primers (5’-CGCGCCATTGTGTTGGTA-3’ and 5’-CATGCATAAGCTTGCTCGAGTCT-3’) were used for PCR amplification. Primers were amino-modified in order to allow a better fixation of the DNA on the coated slides (see below). Two PCR reactions were made in 150µl each to obtain large amounts of DNA and amplification was checked on 1% agarose gel for each PCR. The amplified DNA was subsequently purified under vacuum using the MultiScreen®-PCR plates (Millipore, Basel, Switzerland) to remove PCR buffers and primers, and recovered in 25µl of 10 mM Tris-HCl buffer. The concentration (around 1µg/µ)l was checked on 1% agarose gel.

After arraying the clones in 384-well source plates, 25µl of spotting solution corresponding to the specific coating of the glass slides was added in order to obtain the best printing conditions (see below).

Before dilution of the 600 clones in the spotting solution, 1 µl of purified DNA was diluted in 9 µl of ddH_2O and sequenced using M13 Reverse primers on ABI Prism 377 (PE Biosystems). 10% of the sequences did not give the same sequence as the one in the database but were printed anyway. All the other clones were identical to sequences previously determined from Clemson University or from our laboratory (Appendix 8.1).

2.1.2 Printing device

2.1.2.1 Microarray spotter: GMX417, Affymetrix

Slides were printed using the GMX417 arrayer equipped with a 4-pin/ring print-head from Affymetrix which allows simultaneous production of 42 microarrays. This system needs a relatively high volume of DNA solution as the print-head rings collect 2 µl of the sample that are maintained by surface tension in the rings. The rings have to be submerged within the printing solution which needs a minimum amount of 15 µl of liquid. The pin crosses the ring containing the DNA solution and prints the glass slide. Three hits per spot gave a spot of DNA at high concentration with a regular diameter. To improve the fixing of the DNA on the support, several coatings such as superaldehyde, epoxy, poly-L-lysine, amino-silane, CMT-GAP or FAST are available to allow cross-linking of the DNA strands (Zammatteo et al., 2000; Diehl et al., 2001; Dolan et al., 2001). However, these chemicals need specific printing buffers to obtain a good immobilization of the DNA probes, and, for amine-modified surfaces (poly-L-lysine), a UV treatment is necessary to cross-link the DNA.

2.1.2.2 Slides and coatings

Coating of glass slides is necessary to allow covalent binding of the DNA and to reduce loss of the probes during the different steps of the technique. We have tested superaldehyde-coated slides provided by Telechem International, epoxy- and hydrogelepoxy-coated slides by NoAb

Biodiscoveries (Mississauga, Canada), and poly-L-lysine-coated slides. The coating with poly-L-lysine was made in our laboratory following a Stanford University protocol (http://cmgm.stanford.edu/pbrown/protocols). Microscope glass slides (Erie-Electroverre SA, Romont, Switzerland and Menzel Gläser, Braunschweig, Germany) were cleaned in 10% NaOH/ 60% ethanol solution by mixing for 2 hrs on an orbital shaker. After five washings in ddH2O to remove completely the cleaning solution, slides were transferred to the poly-L-lysine solution (10% poly-L-lysine, 10% PBS) for 20-40 min. Slides were finally washed in fresh ddH_2O, dried by centrifugation 5 min at 500 rpm and then baked at 45°C during 10 min. These slides have to be stored in a dust-free and air-free atmosphere to prevent degradation of the coating layer.

2.1.2.3 Blocking methods

After DNA printing, the free sites need to be blocked prior to hybridisation to prevent fixation of the labelled targets. The superaldehyde slides were washed twice in 0.2% SDS then rinsed twice in ddH_2O and DNA was denatured in boiled water. After drying the slides by centrifugation, the free aldehyde groups were reduced by incubation in a solution of $NaBH_4$ (1 g dissolved in 300 ml PBS) for 5 min. The slides were subsequently washed three times in 0.2% SDS and twice in water, then dried by centrifugation. The epoxy and hydrogelepoxy slides were blocked in NoAb blocking buffer (NoAb Biodiscoveries (Mississauga, Canada)) which contains 25% of SuperBlock (Pierce), and 75% of Tris pH 8.5, ethanolamine 1 mg/ml, glycine 1 mg/ml and lysine 1 mg/ml by pre-incubation 2 hrs before hybridisation. Poly-L-lysine coated slides were chemically deactivated in 200 ml anhydrous 1,2-dichloroethane (DCE, Fluka), 1 g succinic anhydride (Fluka) and 2,5 ml N-methylimidazol (Fluka) (Diehl et al., 2001). After 1 hr of incubation, slides were rinsed in fresh DCE and DNA was denatured 2 min in boiling water. Finally, slides were rinsed in 95% ethanol and dried at room temperature.

2.1.3 Hybridisation

2.1.3.1 Hybridisation test

Before hybridisation with the samples of interest, the quality of the chip was tested. These tests were used to determine the best printing conditions by detecting the presence of DNA in the spots. Therefore, oligonucleotides of random sequences and labelled with the Cyanine-3 and -5 (Cy3, Cy5) dyes were hybridised to the chip. 20 µl of hybridisation mix (3x SSC, 1 mg/ml total yeast RNA, 0.05 M Tris pH 7.4, 0.25% SDS and 150 pmol each of labelled oligonucleotides) were incubated 90 min at 35°C. To remove non-bound oligonucleotides, slides were washed 5 min in 2x SSC, 0.2% SDS, then twice 2 min in 0.05x SSC and subsequently dried by centrifugation (1 min at 1000 g). Slides were then scanned with a ScanArray 5000 (PerkinElmer Life Sciences, Rodgau-Jüdesheim, Germany) (see chapter 2.1.3.4).

2.1.3.2 Target preparation

2.1.3.2.1 RNA extraction

Total RNA of the samples was extracted using the TRizol method. 1g of leaf material was ground in liquid nitrogen and 10 ml of TRizol (38% phenol, guanidine thiocyanate 0.8 M, ammonium thiocyanate 0.4 M, sodium acetate pH 5 0.1 M, glycerol 5%) was added, mixed thoroughly, and incubated for 5 min at room temperature (RT). After centrifugation for 15 min at 12,000 g (4°C), the supernatant was transferred into a new tube and 2 ml of chloroform were added and mixed 15 sec using a vortex. After 2 min incubation at RT, samples were centrifuged for 15 min at 12,000 g (4°C). The supernatant (aqueous phase) was pipetted to a new tube. 0.5 volume of isopropanol and 0.5 volume of 0.8 M sodium citrate/1.2 M NaCl were added and mixed by inversion. After 1hr incubation at -20°C, samples were precipitated by centrifugation (10 min at 10,000 g at 4°C). Pellets were washed in 10 ml 75% ethanol,

centrifuged for 10 min at 10,000 g (4°C), dried and resuspended in 300 ml of DEPC-treated ddH_2O. Samples were subsequently submitted to another purification step using a QIAshredder column (Qiagen) to remove cell debris or sugar that would have co-precipitated with the RNA. Flow-through was carefully collected to avoid the translucent pellet containing the sugars. RNA concentration was determined by OD measurement and samples were stored at -80°C.

2.1.3.2.2 Labelling and purification of the target

Direct labelling adapted from P. Reymond's protocol was used to obtain labelled samples by reverse transcription of the mRNA (Reymond et al., 2000). As the purification of polyA RNA did not result in good yield, total RNA was directly used as template for reverse transcription using a poly-dT primer of 21 nucleotides. 40 µg of total RNA were denatured at 70°C for 5 min together with 2 µg of primer in a total volume of 13 µl. After incubation for 5 min at RT, the reaction mix containing 2 µl of Superscript II, 6 µl of 5x reaction buffer (Invitrogen Life Technologies, Basel, Switzerland), 3 µl of 0.1 M DTT, 0.8 µl of RNase Out 40 U/µl (Invitrogen Life Technologies, Basel, Switzerland), 0.6 µl of 25 mM dATP, dGTP and dTTP, 0.6 µl of 10 mM dCTP and either 3 µl of Cyanine3-dCTP (Cy3) or Cyanine5-dCTP (Cy5) (Amersham, Otelfingen, Switzerland) was added and incubated for 2 hrs at 42°C. After pooling the differently labelled samples, the reaction was stopped and RNA degraded by adding 2.65 µl of 25 mM EDTA and 3.3 µl of 1 M NaOH and incubating for 10 min at 65°C. These salts were neutralised by adding 3.3 µl of 1 M HCl and 5 µl of Tris pH6.8.

Labelled cDNA was purified using the MinElute PCR purification kit from Qiagen (Basel, Switzerland), following the manufacturer's protocol. After subsequent washings in buffers, samples were finally resuspended in 10 µl of DEPC treated water by incubation for 1 min at RT, and collected by centrifugation 1 min at maximum speed. Samples were protected from

light until hybridisation was performed in order to prevent degradation of the light-sensitive dyes.

2.1.3.3 Hybridisation

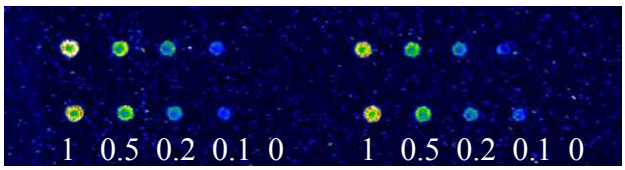
Before hybridisation, 1.25 µl of 10 µg/µl yeast RNA, 1.9 µl 20x SSC and 0.5 µl of 10% SDS were added to the labelled cDNA in order to improve hybridisation quality. The yeast RNA is used to prevent background signals in case of insufficient chemical blocking of the free sites of the microarray and unspecific fixation of labelled cDNA on the slides. The final 3x SSC concentration is allowing good DNA hybridisation and the SDS is essential for a good spread of the mix over the slide by reducing its surface tension.

Labelled samples were added to a cover-slip and the microarray was put onto it and rapidly but carefully inverted and placed into a pre-warmed hybridisation chamber containing 20 µl of 3x SSC in its wells. Chambers were tightly closed and placed in a pre-warmed water-bath for 14-16hrs at 65°C. Washings were then performed under agitation in order to remove unfixed labelled material. Two washings of 6 min in 1x SSC, 0.03% SSC were followed by two washings of 5 min in 0.2x SSC and two washings of 5 min in 0.05x SSC. Removal of SDS is necessary as it could result in background signals.

2.1.3.4 Scanning

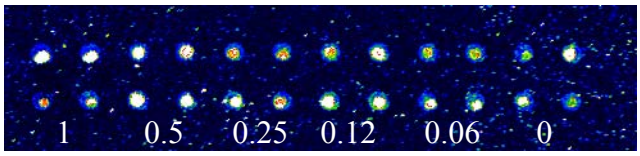
Microarray scanning was performed at the Functional Genomics Centre Zürich with a ScanArray 5000 (PerkinElmer Life Sciences, Rodgau-Jüdesheim, Germany) at 10 mm/pixel resolution. Photomultiplier and laser power were adjusted to obtain similar intensity signals for control spots (house-keeping genes and alien cDNA (Stratagene, Amsterdam, Netherlands)).

A



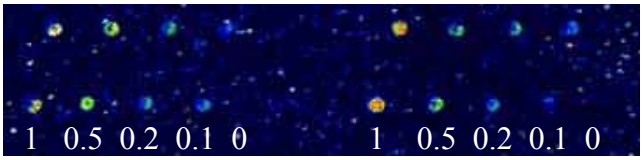
Epoxy slide/Phosphate buffer

B



Superaldehyde slide/ArrayLt buffer

C



Superaldehyde slide/Phosphate buffer

Figure 2: Hybridisation test for the identification of the best printing substrate and buffers adapted to the GMX417 arrayer (Affymetrix). The actin cDNA was diluted between 0 and 1 µg/µl and printed 12 times in duplicate (only two replicates are represented). DNA concentration is indicated below the spots. Printing quality was checked by hybridisation with a Cy3-labelled oligonucleotide. Different substrate/buffer combinations were tested. A: epoxy-coated slide with phosphate buffer, B: superaldehyde slide with ArrayLt buffer, C: superaldehyde slide with phosphate buffer.
The best spots and visible dilution series were obtained with the epoxy-coated slides and phosphate printing buffer. No dilution was visible for the superaldehyde/ArrayLt combination revealing the presence of DNA in this buffer.

2.2 Results

2.2.1 Test chip

A sample of DNA (actin clone) was printed onto different types of coated slides and, after blocking of the free sites, hybridisations with Cy3 or Cy5-labelled unspecific 17mer-oligonucleotides were made to check the quality of the printing. Several slide/buffer combinations were tested to define the best conditions adapted to our arrayer with superaldehyde-, epoxy-, hydrogelepoxy- and poly-L-lysine-coated slides and ArrayLT, betaine, DMSO and phosphate buffers. The buffers interacted differently with the coating substrates resulting in tremendous differences of DNA fixation. The hybridisation test of a dilution series of DNA resuspended in 150 mM sodium phosphate buffer printed on epoxy- or hydrogelepoxy-coated slides clearly resulted in spots with decreasing intensity (Figure 2) whereas the same dilution resuspended in ArrayLT buffer and printed on superaldehyde slides showed the same high intensity for all the spots. Therefore, this combination was not used any further and subsequent tests were made with the phosphate solutions/epoxy and poly-L-lysine slides combinations. In fact, the ArrayLT buffer contained salmon sperm DNA that hybridised also with the Cy3-oligos explaining the bright intensity for all the spots. The presence of the salmon sperm DNA would allow a better spreading of the DNA in the spots but this was not achieved in our conditions.

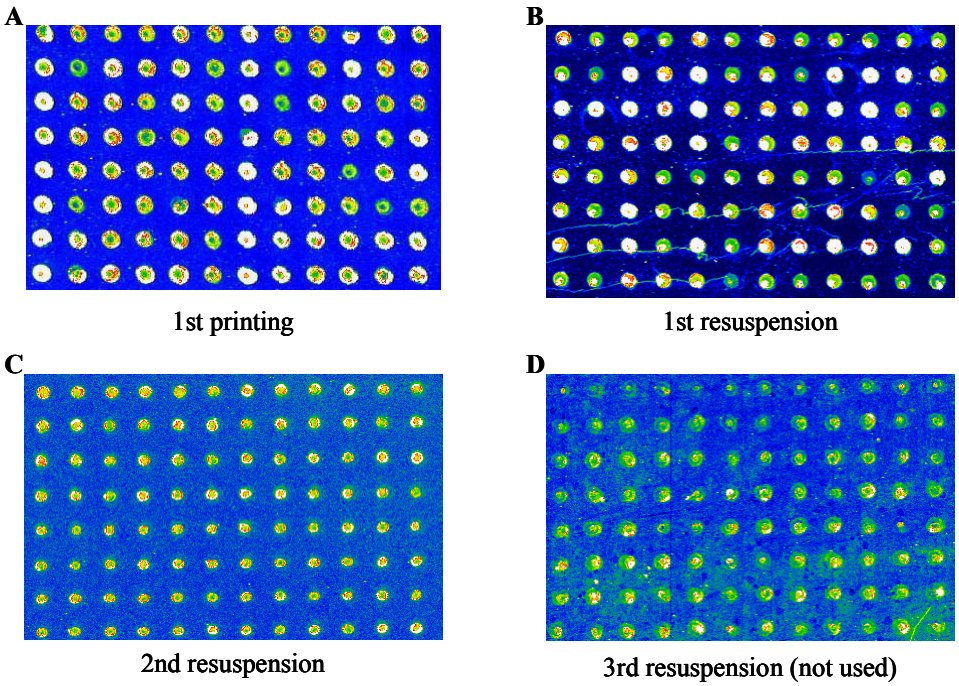


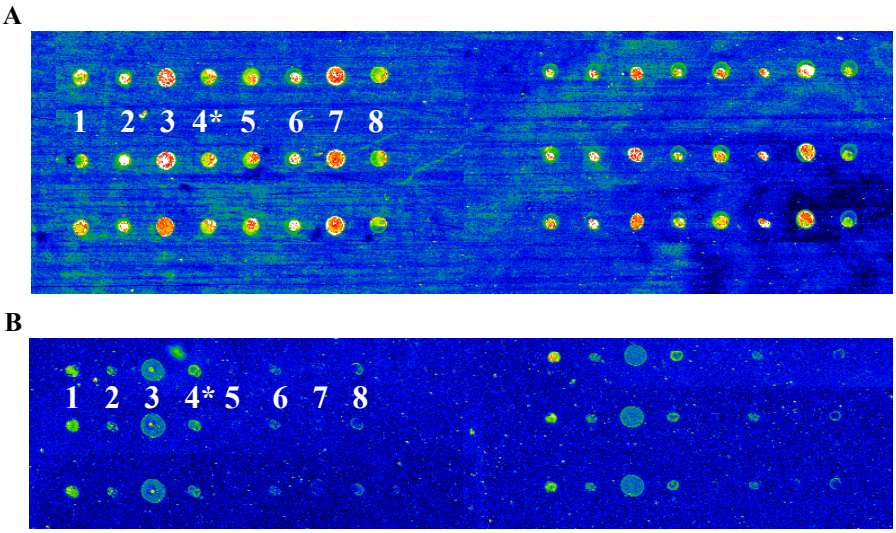
Figure 3: Evolution of the printing quality when using phosphate buffer as spotting solution. Hybridisation of the cDNAs was performed with Cy3-labelled oligonucleotides to check the quality of the printing. A: hybridisation result when the phosphate buffer was added to the freshly prepared cDNAs, B: hybridisation result after a first cycle of drying/resuspension of the templates, C: hybridisation result after two cycles of drying/resuspension, D: after three cycles.
Phosphate salts were hard to dissolve after the drying, reducing the printing efficiency of this solution. DNA could have precipitated with the phosphate salt, decreasing the DNA concentration of the printing solution.

2.2.2 Printing on epoxy-coated slides

The epoxy slides have given the best results in our first attempts. Therefore, the 600cDNA microarray chip was printed on this support. The CO groups of the epoxy coating allow a covalent linkage to the NH_2 groups of the DNA. To print on epoxy-coated slides, the best printing solution is a 150 mM phosphate buffer, pH 8.5 (Figure 3A). This optimizes the attachment of DNA and the spot morphology on the epoxy substrate when freshly added to the DNA. However, this spotting solution evaporated rapidly during the printing in our arrayer, mostly at the borders of the microplates. If the printing solution levels are not the same in the 384-wells microplate, it could result in the absence of printing of some spots/clones. Furthermore, the phosphate salts can precipitate during the storage at -20°C and are hard to resuspend before printing, resulting in spots with bad morphology. To prevent this, the plates were dried after printing and resuspended in ddH_2O ($V = V_{initial} - 2$ µl). However, the phosphate salts were hardly resuspended and this resulted in spots of bad quality after several drying /resuspension steps (Figure 3C and D). Therefore, to reduce the evaporation of the plates, several additives were tested such as betaine and DMSO. Unfortunately, these compounds were not compatible with the epoxy coating (Figure 4). Therefore, in my thesis, these slides were only used for the first printings until a better printing combination was found (Figure 5L to O).

2.2.3 Printing on poly-L-lysine coated slides

An alternative to the epoxy slides has been the use of poly-L-lysine-coated slides. In this case, DNA strands are fixed by UV at the amino groups of the modified surface. Phosphate buffer gave good printing results (Figure 3B, 5A to C). However, better results were obtained when DMSO was added in the printing buffer as can be seen in Figure 4 showing the impact of



1: 50% P buffer
2: 10% DMSO / 50% P buffer
3: 50% DMSO / 50% P buffer
4*: 50%Tris / 50% P buffer
5: 10% DMSO / 50% Tris
6: 10% DMSO / 50% Tris / 40% P buffer
7: 50% DMSO / 50% Tris
8: 50% Tris

Figure 4: Test of eight printing buffers on two different coated surfaces. The cDNA of actin was resuspended in different printing solutions containing DMSO or phosphate (P) in different proportions and printed in six replicates. Quality of the printing was revealed by hybridisation with a Cy3-primer. A: Poly-L-lysine-coated slide, B: Epoxy-coated slide. The buffer highlighted with a star corresponds to the buffer used for the printing of the first 600 cDNA chip. The best results were obtained on the poly-L-lysine substrate with the buffers containing 50% DMSO (3 and 7).

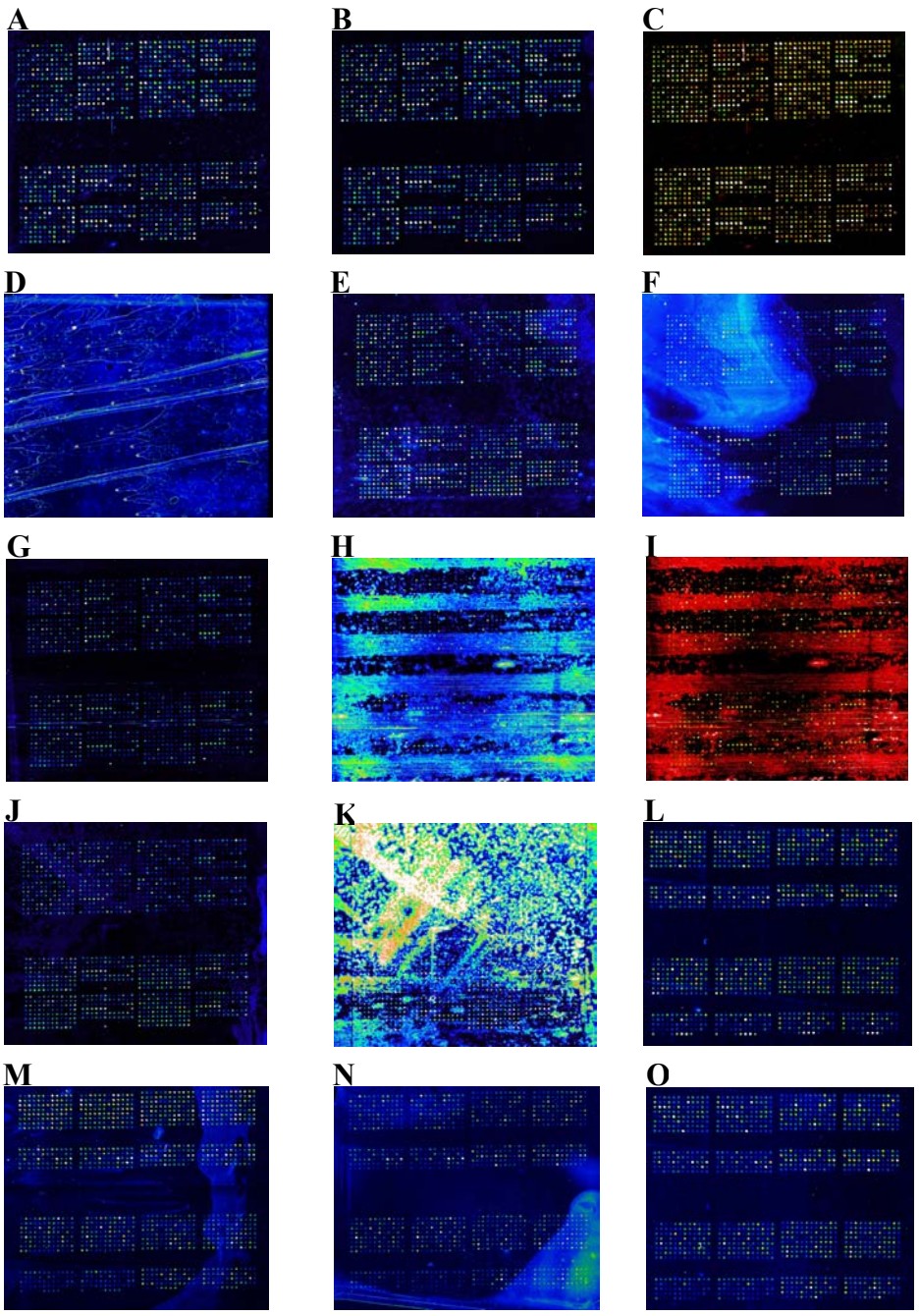


Figure 5: Hybridisation results with RNA-derived labelled targets. A: Perfect hybridisation with a Cy3-labelled target, B: Perfect hybridisation with a Cy5-labelled target on the same slide as in A, C: Overlapping of the pictures A and B in false colour representing highly expressed genes of A in green, highly expressed of B in red and genes with similar hybridisation intensity in yellow, D: Example of background due to the aging of the poly-L-lysine coating surface (Cy3 channel), E: Background in the Cy5 channel but slide still analysable, F: High background in the Cy3 channel preventing the utilisation of the hybridisation results, G: Good hybridisation in Cy3 channel, H: strong background in the Cy5 channel for the same slide as G, I: Overlapping of the pictures G and H, J: As in G with slight background in the top-left corner reflecting the excitement of the Cy5-labelled target (in K), K: As in H, L: perfect hybridisation on Cy3 channel, M: background in the Cy3 channel, N: background in the Cy5 channel, O: perfect hybridisation on the Cy5 channel.
A to F: Erie poly-L-lysine-coated slides G to K: Menzel poly-L-lysine-coated slides, L to O: Epoxy-coated slides.

printing buffers on spot shape and DNA content. Furthermore, this compound reduces the surface tension of the solution and thus decreases evaporation during the printing process. It also denatures double stranded DNA and no denaturation step by boiling is necessary after the blocking of the free sites. Most of the hybridisations of our experiments were performed using this poly-L-lysine slide/DMSO buffer combination. Hybridisation results are represented in Figure 5A to K.

Unfortunately, the blocking process for this type of slides needs highly toxic chemicals (1,2-dichloroethane) which have to be carefully handled. Furthermore, the coating layer can be degraded with time when not correctly stored, resulting in increased background signals (Figure 5D to F). Whereas good microarrays were generally produced using Erie-Electroverre glass slides, strange hybridisation results were obtained when Menzel glass slides were used. In this case, good results were obtained with the Cy3 channel but strong fluorescence on the whole hybridisation surface was detected with the Cy5 channel (Figure 5G to K). Two hypotheses have been elaborated to explain these results. Either Menzel slides are auto-fluorescent in the Cy5 channel or the processing of the Cy5-labelled samples was not correct. In order to detect if the problem came from the RNA samples, RNA which was successfully labelled with Cy3-dCTP was labelled with Cy5-dCTP and reciprocally. Similar results were obtained as previously with strong background in the Cy5 channel only, confirming that the problem was not originating from the RNA samples. The quality of the labelled samples was also checked with a Bioanalyser chip from Agilent (Figure 6) and an additional band of low molecular weight (0.3kb) was present only on the Cy5-labelled samples. This band could correspond to unincorporated Cy5-dCTP that would have co-precipitated with the labelled samples and could bind easily on the chip then producing the strong background.

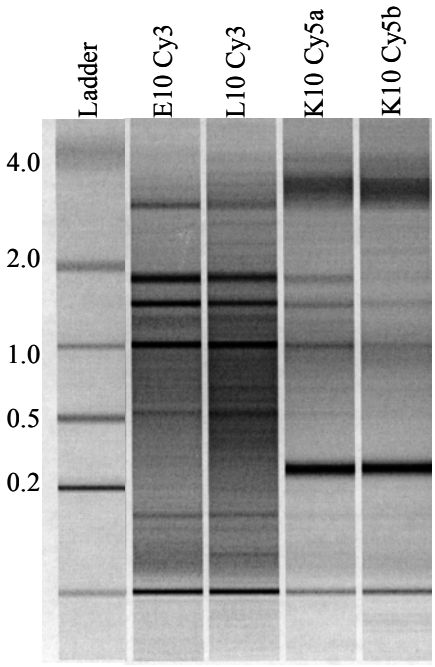


Figure 6: Quality test of labelled cDNA determined with a Bioanalyser chip (Agilent). The ladder scale is in kilobases. E10 Cy3, L10 Cy3: RNA labelled with Cy3-dCTP; K10 Cy5a, K10 Cy5b: RNA labelled with Cy5-dCTP. The samples showed no RNA degradation, the three bands between 1 and 2kb corresponded to rRNAs. Additional bands of 0.3kb and 3kb are present only in the Cy5-labelled samples. The K10 samples had given good hybridisation results without background when labelled with Cy3-dCTP, but gave strong hybridisation background with Cy5-dCTP.

Finally, poly-L-lysine-coated Erie slides have been a good replacement for the epoxy-coated slides. However, in order to obtain good hybridisation results, the production of the chips has to be planned carefully so that the slides are not too old which is the main factor for surface degradation and increased background.

2.3 Conclusion

Setting-up of the microarray technique has been a long process. Finding the right materials adapted to our conditions has taken a long time but good parameters have finally been established to produce microarrays. Thus, most results were obtained on poly-L-lysine coated glass slides using a buffer containing 50% DMSO.

The first printing combination based on epoxy-coated glass slides and phosphate buffer had slowed down our progress to find the “perfect spot”. Epoxy slides resulted in relatively less background than poly-L-lysine-coated slides, but their major problem was the compulsory use of phosphate buffer for printing on this surface. Unfortunately, this buffer was susceptible to high evaporation during printing and salts were difficult to resuspend after drying to homogenise the solution level in each well before the next printing could be performed. Solving this problem was difficult because of the non-compatibility of compounds reducing the evaporation rate (DMSO, betaine) with this buffer. Finally, epoxy slides were abandoned for poly-L-lysine slides. These slides were giving good printing results and allowed to re-use the same template several times without any drying/resuspension cycle as nearly no evaporation of the samples occurred during the printing with the spotting buffer containing DMSO.

III. Specific patterns of changes in wheat gene expression after treatment with three antifungal compounds

Frédérique Pasquer, Edwige Isidore, Jürg Zarn and Beat Keller

2005, Plant Molecular Biology, in press

3.1 Abstract

The two fungicides azoxystrobin and fenpropimorph are used against powdery mildew and rust diseases in wheat (*Triticum aestivum* L). Azoxystrobin, a strobilurin, inhibits fungal mitochondrial respiration and fenpropimorph, a morpholin, represses biosynthesis of ergosterol, the major sterol of fungal membranes. Although the fungitoxic activity of these compounds is well understood, their effects on plant metabolism remain unclear. In contrast to the fungicides which directly affect pathogen metabolism, benzo(1,2,3)thiadiazole-7-carbothioic acid S-methylester (BTH) induces resistance against wheat pathogens by the activation of systemic acquired resistance in the host plant. In this study, we monitored gene expression in spring wheat after treatment with each of these agrochemicals in a greenhouse trial using a microarray containing 600 barley cDNA clones. Defence-related genes were strongly induced after treatment with BTH, confirming the activation of a similar set of genes as in dicot plants following salicylic acid treatment. A similar gene expression pattern was observed after treatment with fenpropimorph and some defence-related genes were induced by azoxystrobin, demonstrating that these fungicides also activate a defence reaction. However, less intense responses were triggered than with BTH. The same experiments performed under field conditions gave dramatically different results. No gene showed differential expression after treatment and defence genes were already expressed at a high level before application of the agrochemicals. These differences in the expression patterns between the two environments demonstrate the importance of plant growth conditions for testing the impact of agrochemicals on plant metabolism.

3.2 Introduction

Plants have evolved effective resistance mechanisms that enable them to defend against pathogen attacks. Nevertheless, all crops are susceptible to a number of major fungal pathogens that cause up to 20% of yield losses (Gullino et al., 2000). In cereals, rusts, mildews and Septoria are the most damaging fungal diseases. In the last decades, a number of systemic fungicides with different modes of action and targets have been developed to reduce the losses caused by these diseases.

Strobilurins form a family of broad-spectrum fungicides that are derived from a natural compound, strobilurin A, which is produced by the wood-rotting fungus *Strobilurus tenacellus* (Bartlett et al., 2002). The synthesis of derivatives of this molecule has led to several active compounds, including azoxystrobin (Gullino et al., 2000). Azoxystrobin and the other strobilurins are inhibitors of fungal mitochondrial respiration by blocking the electron transfer at the Q_0 site of cytochrome bc_1 (Affourtit et al., 2000). Strobilurins currently represent 10% of the fungicide market and are used by farmers to control fungal pathogens such as powdery mildew and rusts. Besides their anti-fungal action, strobilurins are also known for their "greening effect" on the crop which is defined as a delayed leaf senescence and an increased grain-filling period (Bartlett et al., 2002). This side effect seems to result from the inhibition of ethylene biosynthesis by reduction of production of superoxide which is the mediator of the conversion reaction of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene (Wu and von Tiedemann, 2001).

Morpholines are another family of systemic fungicides, known since 1965 (Mercer, 1991). Fenpropimorph was discovered in 1979 and is still commonly used against mildews and rusts. It inhibits two enzymes of fungal sterol biosynthesis (Engels et al., 1998). The morpholine compound inhibits the enzymes sterol Δ^{14} reductase and Δ^{8}-Δ^{7}

isomerase by binding tightly to their catalytic site (Mercer, 1993; Debieu et al., 2000). Some phytotoxic effects like growth delay and altered phytosterol composition have been observed in cereals treated with this fungicide (Mercer et al., 1989; Khalil and Mercer, 1991).

Systemic acquired resistance (SAR) was discovered several decades ago and has been studied intensively (Metraux, 2001). A SAR response leads to pathogen resistance in the whole plant after biological or chemical stimulation. This mechanism allows the plant to protect itself against numerous viral, bacterial or fungal pathogens, depending on the species (Oostendorp et al., 2001). The two main chemical SAR enhancers are 2,6-dichloroisonicotinic acid (INA) and benzo(1,2,3)thiadiazole-7-carbothioic acid S-methylester (BTH), which are both structurally similar to salicylic acid SA (Görlach et al., 1996). In dicotyledonous plants, these molecules induce pathogenesis-related genes and specific genes involved in signalling. Salicylic acid plays a key-role in the signal transduction pathway leading to SAR (Lawton et al., 1995). However, in monocotyledonous plants, the role of SA has not been clearly demonstrated, although the synthesis of SA is induced by aphid damage in barley (Chaman et al., 2003). In wheat, BTH can induce resistance to powdery mildew (*Blumeria graminis*), leaf rust (*Puccinia triticina*) and *Septoria* leaf spot (Görlach et al., 1996) but not to *Fusarium* head blight (Yu et al., 2001). The treatment of wheat plants with SA results in a lower resistance level against powdery mildew compared to plants treated with BTH, suggesting the involvement of other signalling pathway(s) to induce this SAR-like response (Görlach et al., 1996).

The putative effect of these compounds on crops has been tested by studying possible consequences of their primary action (for example with the measurement of sterol content after morpholine treatment (Khalil and Mercer, 1991)) and by few bioassays

(Grossmann and Retzlaff, 1997). However, little is known on their effect on the whole plant metabolism. Genome-wide expression profiling (Schena et al., 1995) is a technology for studying changes of global gene expression after a specific treatment of the plant. For the two closely related species wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), more than 950,000 ESTs have been characterised (see the Triticeae EST database website http://wheat.pw.usda.gov/genome/). Such collections of ESTs can be used for the construction of cDNA microarrays. The putative role of genes of unknown function can be predicted from similarity of expression patterns, even from different species (van Noort et al., 2003). In cereals, this technique has been used in only few studies. Rice cDNA microarrays have been used to study gene expression related to salt stress tolerance of rice (Kawasaki et al., 2001) and iron deficiency in barley (Negishi et al., 2002). Maize development and the response to UV radiation were studied with maize cDNA microarrays (Lee et al., 2002; Casati and Walbot, 2003). In barley, expression profiling was used for the detection of mutated genes (Zakhrabekova et al., 2002) and responses to drought and salt stress (Ozturk et al., 2002) or studying gene-for-gene interaction with powdery mildew using the Barley1 GeneChip from Affymetrix (Caldo et al., 2004).

Extensive toxicological risk assessment on the active compounds of pesticides and on their catabolites in the plant is an integral part of the regulatory approval process of pesticides and a huge database on these compounds is available at World Health Organisation (http://www.who.int/pcs/jmpr/jmpr.htm). Most of the studies concern food safety issues and human health. Therefore, a high food safety level of pesticide residues and their metabolites in crops can be assumed. There is also a broad knowledge on endogenous plant compounds affecting human health (Stegelmeier et al., 1999). In contrast, studies on possible changes in plant metabolism upon pesticide

treatment are not mandatory and there is a lack of information on this aspect for most pesticides.

In order to determine whether the three compounds azoxystrobin, fenpropimorph and BTH alter wheat gene expression, we produced a cDNA microarray containing 600 barley genes covering the major plant biochemical pathways. Here, we report the impact of the two fungicides and the SAR enhancer on gene expression in wheat plants grown under controlled greenhouse conditions and compare these results to a similar trial where plants were grown in an agricultural environment.

3.3 Materials and methods

3.3.1 Plant material and treatments

Seeds of spring wheat (*Triticum aestivum* L., variety Greina) were grown in the greenhouse (16hr light/ 20°C, 8hr night/ 16°C, 2 to 4 seeds per pot). At growth stage 32 (Tottman, 1987), plants were treated with BTH (Bion®, Novartis (Basel, Switzerland) 60g/ha, as recommended by the manufacturer). The second application of Bion® and the spraying of the two fungicides (azoxystrobin, Amistar® from Syngenta (Basel, Switzerland) and fenpropimorph, Corbel® from BASF (Wädenswil, Switzerland) at the concentration of 1l/ha) were made at growth stage 39 (Tottman, 1987). Other plants were kept as untreated controls. In the field, the plants were sown in 5-row plots (1.3 m wide, 1.2 m long, approximately 50 seeds/row) near Zürich, Switzerland, at the Swiss Federal Research Station for Agroecology and Agriculture (FAL Reckenholz, 440m above sea level). For each treatment, 4 plots were sprayed with one fungicide each, following the same protocol as for the treatment in the greenhouse. Four further plots were left untreated and used as control. For both trials, flag leaves were harvested at 24hr, 1 and 2 weeks after treatment.

3.3.2 Preparation of the cDNA microarray

600 cDNA clones of barley (*Hordeum vulgare* L., varieties Morex and CI16155) from our laboratory (SFR clones) and from Clemson University (HVSMEg, HVSMEh and HV_Ceb clones) were chosen to cover major biochemical pathways (for more details about the libraries, see http://wheat.pw.usda.gov/genome for our laboratory's library and http://www.genome.clemson.edu/projects/barley for the Clemson University collection). We used a method adapted from Reymond et al. (2000) to print PCR products amplified from these clones and negative controls (human and *Arabidopsis thaliana* cDNA) onto coated glass slides. Each clone was printed twice. The Clemson clones were amplified twice in 150µl with Taq polymerase (Sigma-Aldrich, Buchs, Switzerland) using 5' end amino-modified M13 universal primers in 35 cycles (94°C, 45s; 52°C, 45s; 72°C, 90s). The SFR clones were amplified in the same volume with the 5' end amino-modified TriplExAmp primers (94°C, 45s; 62°C, 45s; 72°C, 90s) for 10 cycles followed by 25 cycles (94°C, 45s; 55°C, 45s; 72°C, 90s). The DNA products were checked on agarose gels and sequenced with a 377 ABI prism™ DNA sequencer, to confirm their identity. They were concentrated during purification with multiscreen-PCR (Millipore, Volketswil, Switzerland). Two sets of slides were produced, using two different DNA spotters. In the first set, cDNA samples were diluted in print buffer (NoAb Biodiscoveries Inc., Mississauga, Canada) at a concentration of 0.5 to 1µg/µl. PCR products were printed using a GMS417 Arrayer (Affymetrix, Santa Clara, USA) on epoxy-coated glass slides (NoAb Biodiscoveries Inc.). The second set was produced according to the protocol of P. Reymond (http://www.unil.ch/ibpv/WWWPR/Docs/protocols.htm), using the printing facilities of Lausanne University (printing robot (OmniGrid), GeneMachines, Ann Arbor, USA). The purified probes were diluted with a 2X spotting solution (6X SSC, 3M

betain) to the final concentration of 0.5 to 1μg/μl DNA, 3X SSC, 1.5M betain and then printed on QMT Aldehyde slides (Peqlab Biotechnologie Gmbh, Erlangen, Germany).

3.3.3 RNA isolation and preparation of the fluorescent targets

For each treatment and time point, several flag leaves were pooled to reduce biological variation. Total RNA was isolated using TRIzol reagent (Invitrogen Life Technologies, Basel, Switzerland), and 40μg of RNA was used for the reverse-transcription, using either Cy5-labelled dCTP for the treated samples or Cy3-dCTP (Amersham Biosciences, Otelfingen, Switzerland) for the non-treated control samples, adapted from Reymond et al. (2000). The reaction was incubated for 2hr at 42°C. After pooling the treated and control samples, RNA was degraded, the labelled cDNAs were purified using the MinElute PCR purification kit (Qiagen, Basel, Switzerland) and diluted in hybridisation solution (3X SSC, 0.2% SDS, 0.2% yeast tRNA).

3.3.4 Hybridisation on microarrays, scanning and analysis

The target solution was denatured for 1 min at 95°C and applied to the microarray slide which was then covered with a cover slip. Hybridisations were performed in chambers placed in a water bath at 65°C for 14 to16 hr. The slides were washed twice in 1X SSC, 0.03% SDS for 6 min, then twice in 0.2X SSC for 5 min and finally twice in 0.05X SSC for 5 min. They were subsequently dried by centrifugation.

Scanning of the microarray slides was performed using a ScanArray 5000 (PerkinElmer Life Sciences, Rodgau - Jügesheim, Germany) at the resolution of 10μm/pixel. Photomultiplier and laser power settings were adjusted in order to obtain similar intensity levels of signal for the control spots (house-keeping genes and alien

cDNA, Stratagene, Amsterdam, Netherlands) for both channels. Pictures were analysed by Imagene 4.1 software (BioDiscovery Inc., Los Angeles, USA). Spots flagged as empty by the software or manually were removed from the analysis. Normalisation of the signal intensities between the two channels was performed using the global method, and between the slides by scale normalisation (Yang et al., 2002). Experiments with reversion of the dyes did not show significant differences in hybridisation level, so only one way of labelling (control with Cy3 and treated sample with Cy5 dye) was further used. Hybridisations were performed in triplicates, using three different samples treated by the same compound for the three time points. To better assess the gene expression of untreated plants from both growth conditions, microarrays were also performed for the three time points. The samples from the greenhouse were labelled with the Cy5 and those from the field with Cy3. Genes were considered induced or repressed by Significance Analysis of Microarray (SAM, Excel Add-in available at http://www-stat.stanford.edu/~tibs/SAM/). This program allowed the determination of both differentially expressed genes and corresponding false discovery rates (FDR, (Tusher et al., 2001; Hu et al., 2003)). The number of differentially expressed genes and the FDR were determined in order to have one gene considered as falsely detected (Samimi et al., 2005). The obtained FDR were generally below 10% in the greenhouse but higher when there were very few genes differentially expressed after the treatments. Therefore, when only three to six genes out of the 600 genes of the chip were differentially expressed, the FDR were between 16 to 37.5%. Such high ratios have already been observed in a recent study on plant-insect interactions where few genes were differentially expressed and high FDR were obtained (Reymond et al., 2004).

Cluster analyses were carried out using Genesis software (Sturn et al., 2002). Reproducibility between the replicates was checked by measuring correlation and data are presented in trees created with Genesis software.

3.3.5 Northern blot analysis

The results from microarray experiments were partially validated by RNA blot analysis. The same quantity of RNA (40 μg) was electrophoresed and transferred to a nylon membrane as previously described (Feuillet et al., 1997). The labelled probes *WCI2*, *5*, *WIR1c*, actin, *PR 1a/1b* (HV_CEb0006J08f)) were prepared using standard procedures (Sambrook et al., 1989) with clones previously used as templates for the barley cDNA microarray. The RNA blots were analysed using Biomax MS-1 film (Kodak, Lausanne, Switzerland).

3.4 Results

3.4.1 Barley cDNA microarray design

To study gene expression in wheat, we made a cDNA microarray containing 600 cDNAs. Barley and wheat are two species showing high similarity of their gene order (Feuillet and Keller, 2002) and high conservation of gene sequences between the two species (Bennetzen et al., 1998). Several studies have shown that coding sequence identity can reach 100% and is usually over 90% (Ramakrishna et al., 2002; SanMiguel et al., 2002; Caldwell et al., 2004). Therefore, there is good cross-hybridisation between nucleic acids of barley and wheat. Furthermore, as the probes are longer than 400bp, the cDNA microarray allows small inter-species differences with only minimal consequences on hybridisation (Adjaye et al., 2004; Close et al., 2004). The genes used for our chip were chosen from barley (*Hordeum vulgare* cv.

Table 2: Differentially expressed genes in the greenhouse trial 24hr after treatment with either BTH (B), fenpropimorph (F) or azoxystrobin (A). Intensity ratios of genes determined to be differentially expressed by SAM analysis are in bold type. The positive values indicate gene induction and negative values indicate gene repression. The FDR were 5.8%, 4.5% and 10% for B, F and A, respectively.

Gene ID	Accession number	Putative function	B 24hr	F 24hr	A 24hr
wci2	TAU32428	UP|Q41520 (Q41520) Lipoxygenase (Fragment) (EC 1.13.11.12)	**56.7**	**17.8**	1.3
wci1	TAU32427	PIR|T06273 Benzothiadiazole-induced protein clone WCI-1 - wheat	**44.3**	**4.7**	0.9
wci4	TAU32430	homologue to UP|Q41522 (Q41522) Thiol protease	**35.0**	2.2	0.6
HVSMEh0095N14f	BE455009	UP|LOX1_HORVU (P29114) Lipoxygenase 1 (EC 1.13.11.12)	**12.7**	**6.1**	1.2
HV_CEb0006J08f	BE215358	UP|PR1A_HORVU (P32937) Pathogenesis-related protein 1A/1B precursor	**7.3**	**10.7**	**3.0**
HV_CEb0024H14f	BE559397	UP|PR12_HORVU (P35792) Pathogenesis-related protein PRB1-2 precursor	**6.1**	**8.2**	**6.5**
wir232	TATHAU	UP|Q94F70 (Q94F70) Putative thaumatin-like protein	**5.5**	**8.2**	**2.8**
HVSMEg0016C06f[a]	BG344787	similar to UP|COPD_ORYSA (P49661) Coatomer delta subunit	**5.3**	1.9	1.6
wir1c	TARNAWIR1	UP|Q41581 (Q41581) WIR1 protein	**3.9**	**3.0**	**3.2**
HV_CEb0010H13f	BE216428	similar to UP|Q8H841 (Q8H841) Putative receptor-like protein kinase	**3.5**	**2.2**	1.2
HV_CEb0010G19f	BE216411	UP|E13B_HORVU (P15737) Glucan endo-1 3-beta-glucosidase GII precursor	**3.1**	**2.7**	2.7
HV_CEb0003K12f	BE214507	UP|P93180 (P93180) Pathogenesis-related protein 4 precursor	**3.1**	**3.6**	1.0
wci5	TAU32431	PIR|T06278 Benzothiadiazole-induced protein clone WCI-5 - wheat	**3.0**	**2.7**	**2.5**
HVSMEg0005I10f	BG343356	UP|PDI_HORVU (P80284) Protein disulfide-isomerase precursor (PDI) (Endosperm protein E-1)	**2.8**	**3.5**	1.5
wir1b	WHTWIR1PR	UP|WIRB_WHEAT (Q01481) WIR1B protein	**2.8**	**2.3**	**2.7**
HVSMEg0013J19f	BE060855	UP|LX23_HORVU (Q8GSM2) Lipoxygenase 2.3 chloroplast precursor (LOX2:Hv:3) (EC 1.13.11.12)	2.7	**2.9**	0.8
HVSMEh0088H07f	BE195158	UP|PDI_HORVU (P80284) Protein disulfide-isomerase precursor (PDI) (Endosperm protein E-1)	**2.6**	2.3	1.6
HV_CEb0002C16f	BE214080	UP|ENPL_HORVU (P36183) Endoplasmin homolog precursor	**2.6**	2.2	1.8
HV_CEb0003J11f	BE214483	UP|Q43765 (Q43765) Chitinase (EC 3.2.1.14)	2.5	**3.0**	**2.2**
HV_CEb0010L20f	BE216529	UP|PR1_HORVU (Q05968) Pathogenesis-related protein 1 precursor	2.1	**3.6**	2.9
HV_CEb0017B21f	BE558296	similar to UP|Q7XH17 (Q7XH17) Putative receptor-like protein kinase 4	2.1	**2.2**	**1.5**
HV_CEb0003A03f	BE214285	similar to GP|13897320 Somatic embryogenesis receptor-like kinase 2 {*Zea mays*}	1.9	1.7	2.7
HV_CEb0021J19f	BE519892	similar to UP|Q8H8H7 (Q8H8H7) Putative flavanone 3-hydroxylase	1.9	**2.0**	0.9
HVSMEg0011M01f	BE060255	UP|Q9M4C7 (Q9M4C7)Allene oxide synthase (EC 4.2.1.92)	1.6	**3.4**	1.4
HVSMEg0003M07f	AW982621	UP|Q6RYF4 (Q6RYF4) Coatomer alpha subunit	1.6	**2.0**	1.2
HV_CEb0003D01f	BE214349	UP|O65189 (O65189) Glucan endo-1 3-beta-glucosidase	1.4	**2.7**	0.9
HV_CEb0003A01f	BE214283	UP|Q43764 (Q43764) Chitinase (EC 3.2.1.14)	1.4	**1.9**	2.1
HVSMEh0100J22f[a]	BG418805	homologue to UP|FKB7_WHEAT (Q43207) 70 kDa Peptidylprolyl isomerase	1.4	1.2	**2.7**
HV_CEb0003P20f	BE214619	UP|CHS1_HORVU (P26018) Chalcone synthase 1	-1.2	-1.3	**-9.0**

[a]: Clones that have not given the same sequencing result as in the database of Clemson University.

Morex) cDNA libraries from pre-anthesis spikes, spikes 5-45 days after pollination, plants challenged with the powdery mildew pathogen (HVSMEg clones, HVSMEh clones and HV_Ceb clones, Clemson University, respectively) and from our laboratory collection (SFR clones). In blast analyses, we found that the 600 sequences all had corresponding ESTs in the wheat libraries (E value between 0 and e-20 and mean identity level of 90%), reflecting the high homology level between the barley and wheat sequences (see Appendix 8.1). Expression of genes encoding enzymes from the major biochemical pathways of primary and secondary metabolism was analysed. In preliminary tests, wheat derived labelled samples resulted in successful cross-hybridisation with the barley probes on our microarray slides (data not shown). This is in agreement with the generally observed high conservation of coding sequences in wheat and barley (Goff et al., 2002; Close et al., 2004).

3.4.2 Effect of the BTH treatment

The analysis of BTH treated plants grown in the greenhouse revealed that 17 genes were differentially regulated after 24hr (false discovery rate FDR 5.8%), 24 after one week (FDR 4.1%) and 9 after two weeks (FDR 8.9%), which represents about 5 % of the genes present on the chip. All of these differentially expressed genes were over-expressed and none repressed (Figure 7, Tables 2 to 4). The differentially regulated transcripts mainly belong to defence-related genes. Genes encoding glucanase, lipoxygenase and pathogenesis-related genes (PR1a/1b, 2, B1), the wheat induced resistance genes (*WIR1B*, *1C* and *232*) and the wheat chemically induced genes 2 (*WCI2*, lipoxygenase), 1 (*WCI1*, jasmonate-induced), 4 (*WCI4*, protease) and 5 (*WCI5*) showed an increased gene expression of two to nearly 60-fold 24hr after treatment when compared to untreated plants (Table 2). Most of these genes remained

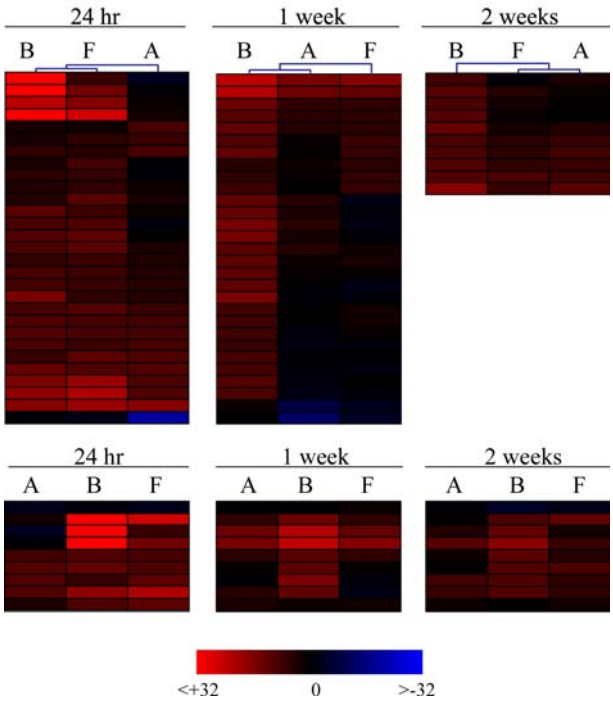


Figure 7: Analysis of gene expression after chemical treatments. Hierarchical clustering of the differentially expressed genes, as displayed by the software Genesis, is shown after treatment with azoxystrobin (A), BTH (B) and fenpropimorph (F) in the greenhouse at the time points 24hr, 1 week and 2 weeks after treatment. The colour scale bar represents the ratio values. Genes with higher expression level after treatment appear in red; those with lower hybridisation intensity appear in blue. After 24hr, the expression patterns of BTH-treated and fenpropimorph-treated plants clustered together (blue tree on top of the clusters). For the subsequent time point, expression profiles of azoxystrobin-treated and BTH-treated plants were more similar to each other, and after two weeks, treatments with fenpropimorph and azoxystrobin resulted in similar differentially expressed genes. The lower panel shows the microarray results of the subgroup of nine genes also analysed by Northern blot (Figure 2). From top to bottom: actin gene, the lipoxygenase gene (WCI2), the thiol protease gene (WCI4), the wheat chemically induced 1 and 5 genes, a wheat induced resistance gene (WIR1c), the pathogenesis related genes PR 1 (HV_CEb0010L20f) and PR 1a/1b (HV_CEb0006J08f) and the protein disulfide-isomerase gene (HVSMEg0005I10f).

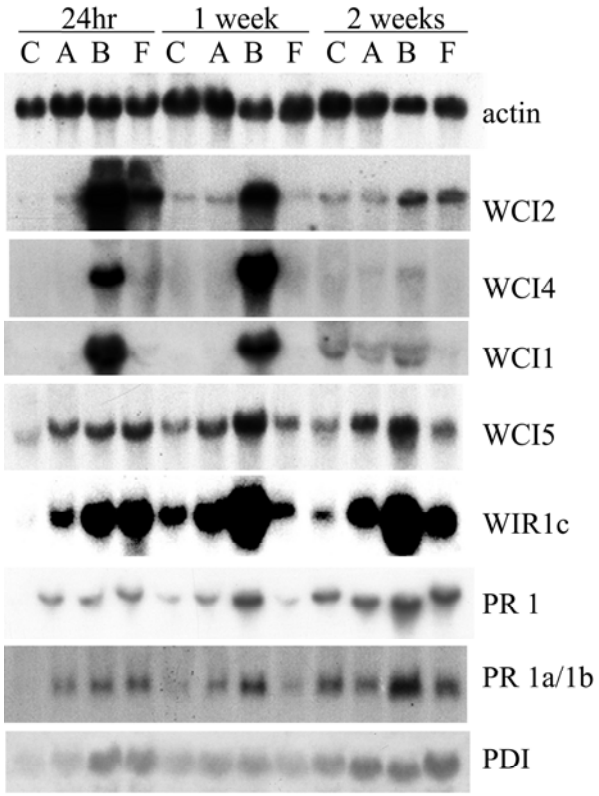


Figure 8: RNA blot analysis showing differential expression 24h, 1 week and 2 weeks after fungicide treatment in the greenhouse trial. C: non-treated control, A: azoxystrobin, B: BTH, F: fenpropimorph. Nine labelled probes were used: the actin gene as quality control of the blots, a lipoxygenase gene (WCI2), three wheat chemically induced genes (WCI4, WCI1 and WCI5), a wheat induced resistance gene (WIR1c), the pathogenesis related genes PR 1 (HV_CEb0010L20f) and PR1a/1b (HV_CEb0006J08f), and the protein disulfide-isomerase gene (HVSMEg0005I10f).

over-expressed one and two weeks after treatment but with decreasing over-expression ratios compared to those obtained at 24hr (Tables 2 and 3). These results were confirmed for some genes (*PR1, PR1a/1b*, *PDI, WCI1, WCI2*, *WCI4, WCI5* and *WIR1c*) by Northern blot analysis (Figure 8) and are in agreement with other studies (Görlach et al., 1996; Stadnik and Buchenauer, 1999). Genes belonging to other functional classes were also up-regulated. Two protein disulfide isomerase genes and one coatomer protein (COP) subunit gene showed an induction of RNA synthesis after 24hr, suggesting an increased biosynthesis of secreted or cell surface proteins (Harter, 1995; Ciaffi et al., 2001). Genes encoding the putative proteins flavanone-3-hydroxylase and caffeoyl CoA O-methyltransferase, involved in flavonoid biosynthesis, showed an activation of transcription only after one week.

3.4.3 Impact of the fenpropimorph treatment

After fenpropimorph treatment, the overall expression pattern was similar to the one obtained after BTH treatment (Figure 7, Appendix 8.2) although seven additional genes (the allene oxide synthase, the putative flavanone 3-hydroxylase, four PR and one putative kinase genes) were significantly over-expressed 24hr after fenpropimorph treatment (FDR 4.5%) compared to the BTH-treated plants (Table 2). For *WCI2* and *1*, the treated/control ratios were lower than the ones obtained with BTH, suggesting a weaker impact of this compound on plant metabolism compared to the SAR enhancer (Tables 2 to 4). Defence-related genes such as the glucanase and PR genes were induced after 24hr but did not show any differential expression later, suggesting a rapid but transient response. The putative thaumatin-like gene (*WIR232*) showed a different pattern, with an induction after 24hr, no differential expression after 1 week but again an increase of mRNA amount after 2 weeks compared to untreated plants. This gene seems to be involved in two phases of the reaction and

could be regulated by a different pathway. Expression of the PR1, PR1a/1b, WCI2, WCI4,

WCI5 and WIR1c and PDI genes were also analysed by Northern blot analyses, giving results similar to the microarray hybridisations (Figure 8). Interestingly, the allene oxide synthase gene was up-regulated after 24hr, suggesting an increase of JA biosynthesis. This indicates that the JA synthesis would be induced after the treatment with fenpropimorph whereas it was not after BTH treatment. As the putative flavanone-3-hydroxylase gene is also induced, the flavonoid synthesis pathway could be triggered early after fenpropimorph treatment.

3.4.4 Effect of the treatment with azoxystrobin

The strobilurin fungicide is known to produce a "green effect" on wheat plants as non-fungicidal secondary effect, with darker green leaves, enhanced concentration of chlorophyll and increased biomass production (Grossmann and Retzlaff, 1997). It also induces some antioxidant activity (Wu and von Tiedemann, 2002). After azoxystrobin treatment, only few genes showed an alteration of their expression, with a high FDR of 10, 16 and 37.5% as there were only ten, four and three genes differentially expressed after 24hr, one and two weeks, respectively (Tables 2 to 4). The PR1, 1A/1B and B1-2 genes were significantly over-expressed after 24hr only, and the WCI1 gene after one and two weeks. The expression levels of *WCI5, WIR1b* and *WIR1c* were similar to each other with a small up-regulation detected by microarray analysis only 24hr after treatment. No statistically significant induction of these genes was observed with microarray analysis for the last two time points. However, over-expression of *WCI5* and *WIR1c* was detected for all time points by Northern analysis (Table 2, Figure 8). Interestingly, the wheat chemically induced genes 4 and 2 that had the strongest up-regulation after BTH and fenpropimorph treatment were not

Table 3: Differentially expressed genes in the greenhouse trial one week after treatment with either BTH (B), fenpropimorph (F) or azoxystrobin (A). Intensity ratios of genes determined to be differentially expressed by SAM analysis are in bold type. The positive values indicate gene induction and negative values indicate gene repression. The FDR were 4.1%, 16% and 25% for B, F and A, respectively.

Gene ID	Gene accession	Putative function	B 1 week	F 1 week	A 1 week
wci1	TAU32427	PIR|T06273 Benzothiadiazole-induced protein clone WCI-1 - wheat	**13.2**	**6.3**	**5.1**
wci4	TAU32430	homologue to UP|Q41522 (Q41522) Thiol protease	**11.7**	**3.8**	**4.4**
HV_CEb0010L20f	BE216529	UP|PR1_HORVU (Q05968) Pathogenesis-related protein 1 precursor	**5.4**	-1.2	-1.1
HV_CEb0024H14f	BE559397	UP|PR12_HORVU (P35792) Pathogenesis-related protein PRB1-2 precursor	**5.3**	-1.3	1.8
wir232	TATHAU	UP|Q94F70 (Q94F70) Putative thaumatin-like protein	**4.9**	-1.1	1.4
wci2	TAU32428	UP|Q41520 (Q41520) Lipoxygenase (Fragment) (EC 1.13.11.12)	**4.5**	2.0	2.0
HV_CEb0003K12f	BE214507	UP|P93180 (P93180) Pathogenesis-related protein 4 precursor	**3.9**	-1.2	1.5
HV_CEb0010N06f	BE216563	homologue to GP|17981573 Kinase R-like protein {*Triticum aestivum*}	**3.8**	2.1	1.1
HV_CEb0006J08f	BE215358	UP|PR1A_HORVU (P32937) Pathogenesis-related protein 1A/1B precursor	**3.8**	-1.3	1.7
HVSMEh0095N14f	BE455009	UP|LOX1_HORVU (P29114) Lipoxygenase 1 (EC 1.13.11.12)	**3.7**	1.8	1.7
HVSMEg0002G09f	AW982228	homologue to UP|Q75RZ2 (Q75RZ2) Putative caffeoyl CoA O-methyltransferase	**3.7**	1.4	1.2
HV_CEb0021J19f	BE519892	similar to UP|Q8H8H7 (Q8H8H7) Putative flavanone 3-hydroxylase	**3.5**	-1.4	-1.0
wir1c	TARNAWIR1	UP|Q41581 (Q41581) WIR1 protein	**3.5**	1.1	1.1
wci5	TAU32431	PIR|T06278 Benzothiadiazole-induced protein clone WCI-5 - wheat	**3.3**	1.9	1.8
HV_CEb0010G19f	BE216411	UP|E13B_HORVU (P15737) Glucan endo-1 3-beta-glucosidase GII precursor	**3.3**	1.3	1.6
HV_CEb0011F02f[a]	BE216529	UP|PR1_HORVU (Q05968) Pathogenesis-related protein 1 precursor	**3.0**	-1.0	-1.2
HV_CEb0015B10f	BE558194	weakly similar to GP|22535653 Putative protein kinase Xa21 receptor type precursor {*Oryza sativa*}	**3.0**	**2.3**	1.1
HV_CEb0011F16f	BE216724	homologue to UP|Q8W4U9 (Q8W4U9) Clathrin assembly protein AP17-like protein	**2.9**	-1.1	-1.4
HV_CEb0011I04f	BE216768	similar to UP|Q84S61 (Q84S61) Putative serine/threonine kinase protein	**2.7**	-1.1	-1.1
HVSMEh0088K24f	BE195244	UP|Q42839 (Q42839) Chitinase (EC 3.2.1.14)	**2.6**	-1.2	-1.1
HV_CEb0010N04f	BE216561	homologue to UP|Q41328 (Q41328) Pto kinase interactor 1	**2.5**	1.3	1.0
HV_CEb0017B21f	BE558296	similar to UP|Q7XH17 (Q7XH17) Putative receptor-like protein kinase 4	**2.4**	-0.9	-1.2
HVSMEg0007A12f	BG343889	similar to UP|Q6J2K7 (Q6J2K7) Protein tyrosine phosphatase	**2.4**	-0.8	-1.0
HVSMEh0099N09f[a]	BE601871	homologue to UP|TBP2_WHEAT (Q02879) TATA-box binding protein 2	2.3	**2.1**	1.0
HV_CEb0010P06f	BE216610	homologue to UP|Q43220 (Q43220) Peroxidase (EC 1.11.1.7)	**2.1**	-1.2	-1.3
HVSMEh0102L08f	BE603237	similar to UP|Q8S8Z0 (Q8S8Z0) Protein phosphatase 2C	1.9	**2.0**	1.1
HVSMEh0081G18f	BE193520	similar to SP|P37837 Transaldolase (EC 2.2.1.2)	1.2	-1.5	**-2.5**
HVSMEh0081M04f	BE193575	homologue to GB|CAA75793 Sucrose synthase 2 {*Hordeum vulgare* subsp. *vulgare*}	-1.1	-1.6	**-3.9**

[a]: Clones that have not given the same sequencing result as in the database of Clemson University.

induced after 24hr but showed up-regulation after one and two weeks, respectively. This indicates that the responses after the application of azoxystrobin are only partially overlapping with the pattern generated by the two other compounds.

A chalcone synthase gene showed a high repression level 24hr after treatment. This gene, like the lipoxygenase genes, is regulated by ethylene (Wan et al., 2002). The antioxidant and "green" effects of azoxystrobin are attributed to a reduction of ethylene production (Grossmann and Retzlaff, 1997). Thus, the reduction of chalcone synthase (HV_CEb0003P20f) expression as well as the absence of over-expression of the three lipoxygenase genes (*WCI2*, HVSMEg0013J19f and HVSMEh0095N14f) after 24hr confirm that azoxystrobin has a specific effect on wheat gene expression which is different from the action of fenpropimorph and BTH (Table 2). After one week, two genes involved in sugar metabolism (a gene similar to a transaldolase gene and a sucrose synthase 2 gene) were down-regulated.

3.4.5 Impact on gene expression of the plant protection compounds in the field

Fungicide and BTH treatments were made in the field to compare gene expression with plants treated in the greenhouse. Surprisingly, no gene showed differential expression after treatment of the plants in the field, whatever the compound used. The WCI2 gene showed higher expression in the Northern blot 24hr after BTH treatment (Figure 9) but this was not statistically significant in the microarray experiments. The absence of differentially expressed genes in the field trial was supported by a high reproducibility between replicates (see Appendix 8.3). The non-treated plants showed a high expression of the defence-related genes that were over-expressed after the treatments in the greenhouse trial (Figure 9 and Appendix 8.4). For all three time points, the PR1, PR1a/1b, WIR232, WIR1c, a β1-3 glucanase, two peroxidase and four chitinase genes were significantly higher expressed compared to the plants grown

Table 4: Differentially expressed genes in the greenhouse trial two weeks after treatment with either BTH (B), fenpropimorph (F) or azoxystrobin (A). Intensity ratios of genes determined to be differentially expressed by SAM analysis are in bold type. The positive values indicate gene induction and negative values indicate gene repression. The FDR were 8.9%, 20% and 37.5% for B, F and A, respectively.

Gene ID	Accession number	Putative function	B 2 weeks	F 2 weeks	A 2 weeks
wci1	TAU32427	PIR|T06273 Benzothiadiazole-induced protein clone WCI-1 - wheat	**6.4**	**2.2**	**3.3**
wci4	TAU32430	homologue to UP|Q41522 (Q41522) Thiol protease	**4.0**	1.4	1.6
wci5	TAU32431	PIR|T06278 Benzothiadiazole-induced protein clone WCI-5 - wheat	**3.4**	1.6	1.2
wir1c	TARNAWIR1	UP|Q41581 (Q41581) WIR1 protein	**3.2**	1.1	1.0
wir232	TATHAU	UP|Q94F70 (Q94F70) Putative thaumatin-like protein	**3.2**	**2.9**	**2.4**
HV_CEb0006J08f	BE215358	UP|PR1A_HORVU (P32937) Pathogenesis-related protein 1A/1B precursor	**3.1**	1.9	2.2
HV_CEb0010L20f	BE216529	UP|PR1_HORVU (Q05968) Pathogenesis-related protein 1 precursor	**2.9**	1.9	2.5
wci2	TAU32428	UP|Q41520 (Q41520) Lipoxygenase (Fragment) (EC 1.13.11.12)	2.9	**3.9**	**2.7**
HV_CEb0024H14f	BE559397	UP|PR12_HORVU (P35792) Pathogenesis-related protein PRB1-2 precursor	**2.7**	1.7	2.1
pBTag	AJ237942	UP|Q9SM34 (Q9SM34) Putative germin-like protein precursor	**2.4**	-1.1	1.4

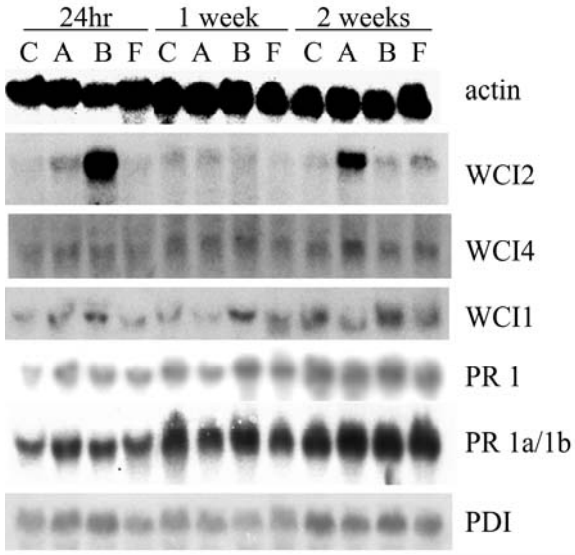


Figure 9: RNA blot analysis showing differential expression 24h, 1 week and 2 weeks after fungicide treatment in the field trial. C: non-treated control, A: azoxystrobin, B: BTH, F: fenpropimorph. Seven labelled probes were used: actin gene as quality control of the blots, a lipoxygenase gene (*WCI2*), and the wheat chemically induced genes (*WCI4*, *WCI1* and *WCI5*), the pathogenesis related genes PR 1 (HV_CEb0010L20f) and PR 1a/1b (HV_CEb0006J08f). 24hr after the BTH treatment and two weeks after the azoxystrobin treatment, the WCI2 gene showed high expression but in microarray experiments this was not statistically significant and only found in one of the three replicate slides.

in the greenhouse. Other defence-related genes were also more expressed in the field for one or two time points (PR4, WIR1a, β1-3 glucanase, glutathione peroxidase, two chalcone synthase, two kinase genes). These results suggest that many of the genes induced by these fungicides in the greenhouse are constitutively expressed during growth in the agricultural environment. However, not all the defence-related genes did behave similarly. The WCI 1, 2 and 4 genes did not show any significant changes in expression between the two growth conditions (Appendix 8.4). The alteration of response to the plant protection compounds could be due to a combination of stresses occurring in the field (Rizhsky et al., 2002). In contrast to the genes over-expressed under field conditions, some genes like the RNase S-like protein gene or the inositol-3-phosphate synthase gene were more expressed in the greenhouse than in the field (Appendix 8.4). However, the apparent over-expression of the RNAse S-like gene could be due to the time-shift when collecting the samples (morning in the greenhouse and afternoon for the field experiments) as it has been shown that this gene is light responsive (Gausing, 2000).

3.5 Discussion

A SAR enhancer and two commonly used fungicides with different and specific chemical modes of action were used in this study to better understand their impact on wheat gene expression and plant metabolism using cDNA microarrays. A relatively small proportion of the genes present on the chip (around 5%) showed differential expression after the treatments. Although few transcripts showed differential expression profiles, common and compound-specific patterns were observed after the three different treatments. Most of the differentially expressed transcripts detected belonged to defence-related gene families, such as the PR and WIR genes, revealing that fungicides can have not only an impact on their fungal targets but have also an

impact on the plant itself more or less similar to BTH. Interestingly, no gene coding for key-enzymes of the primary metabolism showed differential expression pattern after BTH and fenpropimorph treatments, showing that these compounds mainly affect specific sets of genes and defence pathways. Only two genes belonging to the sugar metabolism pathway and coding for sucrose synthase 2 and transaldolase, respectively, were down-regulated one week after azoxystrobin treatment.

BTH, fenpropimorph and azoxystrobin induced genes known to be involved in plant defence against pathogens. The PR1 and wheat induced resistance genes (*WIR1b, WIR1c, 232*) were activated early after the treatment with these compounds, following a similar pattern as in *Arabidopsis* and tobacco after BTH treatment (Friedrich et al., 1996; Lawton et al., 1996). However, the induction of the PR1 genes after BTH treatment contradicts previous results (Molina et al., 1999; Yu et al., 2001) where no activation of these genes was observed, but is in agreement with the results of Görlach et al. (1996). Thus, the induction of the PR1 genes after stimulation by BTH seems to depend on wheat variety, developmental stage or growth conditions of the plant.

After BTH treatment, the wheat chemically-induced genes (*WCI2, WCI1, WCI4* and *WCI5*) were strongly induced as previously described (Rebmann et al., 1991; Bull et al., 1992; Görlach et al., 1996; Mauch et al., 1997). These genes were also induced after the application of fenpropimorph although the levels of differential expression were not as high as after the BTH treatment. In addition, genes like the PDI and COP genes were also induced after BTH and fenpropimorph treatments. Their expression in wheat has been described during plant development (Ciaffi et al., 2001) but not after chemical treatment. These results might indicate that BTH and fenpropimorph induce the secretory and cell surface protein biosynthesis machinery. The activation of the PDI genes could play a role in signal transduction, as the enzyme can break

disulfide bonds that can subsequently allow the monomerisation of a key component of this pathway, as described for NPR1 in *Arabidopsis* (Mou et al., 2003).

In dicotyledons, SA plays an essential role in pathogen resistance as plants defective in SA synthesis cannot develop a SAR response (Lawton et al., 1995). However, in monocots, SA is probably less important, as treatment with this molecule only resulted in mild resistance against fungal pathogens and low induction of genes that are over-expressed after BTH application (Görlach et al., 1996). In our experiment, the ethylene and jasmonate pathways seem to be important for triggering SAR as the *WCI2* and *WCI1* genes (encoding lipoxygenase and jasmonate-induced protein, respectively) showed the highest level of over-expression after BTH and fenpropimorph applications.

Interestingly, the morpholine fungicide qualitatively induced a very similar expression pattern in wheat as BTH, albeit at a quantitatively lower level. Furthermore, the changes induced by this sterol biosynthesis inhibitor were more transient, as fewer differentially expressed genes were observed after one and two weeks. The allene oxide synthase gene was over-expressed after 24hr. This gene encodes one of the key enzymes of jasmonic acid synthesis. This molecule could enhance the defence responses via an increase of the phenylpropanoid pathway metabolism. As morpholine seems to have a relatively mild effect on gene expression, the previously observed negative growth effects on plants (Mercer et al., 1989) could be due to the inhibition of major sterol biosynthesis or to a repression of the soil microorganisms after treatment with fenpropimorph (Thirup et al., 2001).

The azoxystrobin treatment slightly increased the expression of PR genes after 24hr but not the expression of lipoxygenase genes that are known to be induced by ethylene. Furthermore, and in contrast to the other two treatments, down-regulation of

the chalcone synthase gene was observed 24hr after the treatment. This gene is activated by ethylene and its corresponding protein is involved in the biosynthesis of phytoalexins. The lack of expression of this gene might indicate that ethylene synthesis and/or signalling is repressed after azoxystrobin treatment (Schenk et al., 2000). Thus, lower ethylene levels could explain the lower induction of defence-related genes after azoxystrobin treatment compared to the results obtained after the BTH and fenpropimorph treatment.

Our data demonstrate that the morpholin fungicide obviously not only acts on the pathogen metabolism (Engels et al., 1996; Rohel et al., 2001) but can also lead to the induction of a similar set of plant defence genes as the SAR enhancer BTH. Such secondary effects of fungicides might be beneficial for the plants by inducing defence mechanisms before pathogen attack. The effectiveness of another fungicide, fosetyl, is dependent on the SAR pathway in *A. thaliana* and this compound may trigger the SAR because of its phytotoxicity (Molina et al., 1998). The same phenomenon could also explain the induction of the defence-related genes after the application of morpholin on wheat. The plant defence response and the direct fungicidal activity might both synergistically contribute to the observed action of morpholin against pathogens (Molina et al., 1998). The azoxystrobin treatment appears to have a weaker impact on defence-related gene expression because of ethylene inhibition but still some slight induction of PR genes occurred, probably due to other signalling molecules. The lower ethylene level could affect the defence response of the plant and could explain the lower fungicidal activity of azoxystrobin against some wheat pathogens if compared to other compounds (McCabe et al., 2001). Similarly, strobilurin was also shown to induce resistance against several pathogens in tobacco but reduced the hypersensitive response (Herms et al., 2002). In parallel, Arabidopsis

mutant insensitive to ethylene (*ein2*) showed either susceptibility or enhanced resistance to different pathogens (Kunkel and Brooks, 2002). Combinations of azoxystrobin with other fungicides (morpholin or triazole) on winter wheat resulted in better yield (McCabe et al., 2001). The treatment with these other fungicide compounds could counteract the effects of reduced ethylene synthesis.

In our experiments, the differences in gene expression patterns between the individual compounds suggest that BTH is a strong trigger of signalling mediated by ethylene and probably SA. Fenpropimorph seems to activate these pathways less strongly but triggers also the JA signalling pathway, whereas azoxystrobin potentially could induce slightly the JA and SA response pathways but inhibit the ethylene pathway. More experiments are needed to confirm these hypotheses as it is well known that cross-talk exists between these signal transduction pathways (Glazebrook, 2001; Kunkel and Brooks, 2002). The study of the early effects of these fungicides (before 24hr) should also help to determine the primary targets, receptors and first steps of the signal transduction pathways involved specifically in wheat depending on the compound applied. The application of strobilurin on *Arabidopsis* mutants in the SAR signal transduction pathways could also help to understand these complex mechanisms more precisely. Strobilurins apparently affect ethylene production by inhibiting induction of the ACC synthase at the post-transcriptional level (Grossmann and Retzlaff, 1997). The specificity of this inhibition might allow a more precise identification of the role of ethylene in this phenomenon.

The induction of defence-related genes by fungicides was surprising and raised some questions about the function of these genes. It is likely that some of them could be not only defence genes against pathogens but contribute to the induction or increase of some metabolic pathways that lead to the resistance of the plant against diverse

environmental and biochemical stresses (Wan et al., 2002). E.g., they might play a general role in the restoration of the cellular homeostasis.

Gene induction by fungicides and BTH differed dramatically in the field when compared with the greenhouse trial. Plants grown in an agricultural environment are constantly subjected to combinations of stress (drought, wind and pathogen attacks) and our results showed for the three analysed time points expression of a very similar set of defence-related genes as after BTH treatment, except for the WCI genes. The impact on gene expression of the three plant protection compounds in the field environment was barely observable and even BTH, which is the strongest enhancer of defence-related genes, did not trigger the transcription of the SAR markers as in the greenhouse trial. The WCI genes seemed to be induced by BTH only under greenhouse conditions. This was also confirmed in other field experiments (R. Dudler, personal communication). Probably, stress combinations (pathogen attack or heat shock) in the field had specifically induced defence-related genes. Consequently, the transcriptional machinery might have been altered and the chemically induced genes could not be induced anymore by BTH. This phenomenon could reflect the transcriptional memory of the plant which responds differently according to consecutive stresses, i.e. plants show different expression patterns when submitted to either one type of stress or to a consecutive combination of stressful events (Rizhsky et al., 2002; Voelckel and Baldwin, 2004). Therefore, the WCI gene expression could have been suppressed by a response to a previous stress event in the field. In a similar manner, the expression of catalase and peroxidase genes is suppressed when drought and heat shock are both applied in tobacco whereas they are over-expressed in the case of a unique stress (Rizhsky et al., 2002). It has been shown that treatment with BTH in the field induced resistance of wheat against powdery mildew (Görlach et al.,

1996). This resistance is possibly triggered by genes not present on our chip or genes expressed at very low level. It could also originate from post-transcriptional changes in gene or protein activity. Strobilurin and morpholin apparently did not induce genes of any defence-related signal transduction pathway in the field.

Our data demonstrate the importance of the environmental growth conditions when testing the effect of agrochemical products on plants, e.g. in studies related to food safety aspects of pesticide treated crops. It is interesting to note that there are few studies on putative changes of plant metabolism induced by pesticide application. This is in great contrast to the analysis of genetically modified plants where possible changes in plant metabolism are one of the cornerstones in safety assessment.

3.6 Acknowledgments

We would like to thank Geri Herren and Anselme Fournier for their technical assistance, Beatrice Senger and Dr. Gabriele Schachermayr for their help with field experiments. We thank Dr. Nils Stein (IPK, Gatersleben) and Dr. Philippe Reymond (University of Lausanne) for their help during the set-up of the microarray technique in the laboratory. This work was supported by the Swiss Federal Office of Public Health, contract no. 01.000004 (Reg. 2.26.02.-14).

IV. Comparison of wheat gene expression in the greenhouse and field using two microarray platforms.

4.1 Introduction

In chapter III, large differences in gene expression after fungicide application between field and greenhouse plants were described: whereas 10% of the genes of our chip had a differential expression pattern in the greenhouse trial, no gene was differentially expressed in the field after the fungicide treatments. After parallel hybridisations of the controls from the greenhouse and the field on the same cDNA microarray chip, we discovered that the differences of these expression patterns were mainly due to the different environments during plant growth (with a higher expression of defence-related genes in field-grown plants compared to greenhouse-grown plants, see chapter III).

Recently, a new GeneChip from Affymetrix based on barley cDNA sequences has been released (Close et al., 2004). A first study on barley-mildew interaction using this chip was recently published (Caldo et al., 2004). Gene expression of three near-isogenic barley lines infected with either virulent or avirulent powdery mildew races was assessed with the Barley 1 GeneChip. Several genes involved in plant defence and possibly in pathogen recognition were identified by specific transcript accumulation a few hours after inoculation. The high homologies between cereal species allowed cross-hybridisation experiments between barley and wheat (Close et al., 2004). As we were using a small chip containing only 600 barley genes (chapter III), we wanted to investigate the transcription profile of plants grown under different conditions (greenhouse and field) with a chip containing a larger set of genes. The barley GeneChip array contains 22,841 oligonucleotide probe sets of 25bp. From these probes, 22,791 are barley-derived sequences and 48 correspond to the Affymetrix spiking control genes of bacterial or eukaryotic origin. For the Barley1 chip, each gene is represented by 11 pairs of perfect match (PM) probes designed from the 600 last nucleotides of each cDNA contig. Mismatch (MM) probes with the 13^{th} nucleotide different from the PM probe were also designed in order to distinguish non-specific hybridisation from low level

hybridisation (Lipschutz, 1999; Irizarry et al., 2003). Probes of one set are distributed randomly on the GeneChip to avoid putative local defect problems (such as local damages, scratches, dust), but the PM probe and its corresponding MM oligonucleotide are always next to each other (Liu et al., 2002).

The first aim of this experiment was to confirm the use of the barley GeneChip for gene expression studies in wheat. In the Affymetrix chip, probes are derived from the 3'- UTR region and wheat sequences may be more divergent in this region compared to coding sequences. This might result in lower hybridisation with wheat-derived samples than with barley-derived cRNA (Close et al., 2004). Nevertheless, Close et al. (2004) have already shown that wheat-derived samples can successfully hybridise on the Barley1 GeneChip but to a lower degree compared to barley samples (23% of present calls against 43%, respectively). In this study, some probes hybridised with the wheat samples but not with the barley ones, suggesting differences in gene expression between the two species. We also wanted to confirm and extend the results we had already obtained with our cDNA array about the impact of the growth conditions on the gene expression pattern using the Affymetrix GeneChip. This should lead to the identification of additional genes that are up- or down-regulated in the two environments. Ultimately, this should reveal the most important biochemical pathways that are involved in the adaptation to different environments. Such data could then be used as a reference for future studies on the fitness of wheat varieties under different growth conditions and could form a good basis to determine genes specifically involved in such traits.

4.2 Material and methods

4.2.1 Plant material, RNA extraction and labelling of the probes for the cDNA microarray system

Flag leaves of spring wheat plants (variety Greina), grown in the greenhouse under controlled conditions (photoperiod of 16h, 18-22°C) and in the field (collection time in June 2002) from growth level BBCH 32, were collected and directly frozen in liquid nitrogen. Total RNA from six samples (three from the greenhouse, three from the field, see chapter III for details on the field growth conditions) was isolated using the TRizol method, Invitrogen. Quality was checked using the RNA Nano LabChips® on the 2100 Bioanalyzer, Agilent Technologies.

All the material and methods used for the cDNA chip were described in chapter III.

4.2.2 Labelling of the probes for the Affymetrix system

The labelling process for studying gene expression using the Affymetrix GeneChip differs from the one used for cDNA microarray labelling. First, only one sample can be hybridised on this chip whereas two targets can be jointly hybridised on the cDNA microarray. Secondly, RNA samples are first reverse-transcribed, a second strand is synthesised and then cRNA is made using the double stranded cDNA as template to be hybridised on the chip.

First strand cDNA was synthesised starting from 15 µg of total RNA and 100 pmol of T7-(T)24 primer (GGCCAGTGAATTGTAATACGACTCACTATAGGAGGCGG(dT)24) and 500 U of Superscript II reverse-transcriptase and buffers, salts and dNTP according to the manufacturer (cDNA synthesis kit from Invitrogen, Basel, Switzerland) during one hour incubation at 42°C. The second strand was synthesised with the same kit using the previous mix but with specific enzymes (10U DNA ligase, 40U DNA polymerase) and buffers, salts and dNTP during 2 hr-long incubation at 16°C. T4-DNA polymerase was then added and incubated for 5 min. The reaction was stopped by adding 10 µl of 0.5 M EDTA. The double

stranded cDNA was purified by centrifugation in 25:24:1 phenol:chloroform:isoamylalcohol using the Phase Lock Gel™ Light, Eppendorf (Basel, Switzerland); the cDNA was then precipitated in 2.5 volumes ethanol/ 0.5 volume 7.5 M ammonium acetate and resuspended in 12 µl DEPC treated H_2O. Biotinylated cRNA was finally obtained by *in vitro* transcription of 5 µl of the cDNA template using the Bioarray High yield RNA Transcript Labelling kit from Enzo/Affymetrix and purified with the RNeasy columns from Qiagen (Basel, Switzerland). Quality was checked again with the RNA Nano LabChips® on the 2100 Bioanalyzer. The cRNA was then quantified using the following formula:

$$\text{adjusted cRNA} = \text{cRNA}_{\text{measured}} - (\text{total RNA}_{\text{initially taken}}) \text{ (fraction of cDNA used)}$$

Yields between 1.1 and 1.8 µg/µl were obtained for all the samples and the purity ratio (260/280) was between 1.9 and 2.1. Prior to hybridisation, samples were fragmented using the fragmentation buffer from the GeneChip Sample Cleanup Module (Affymetrix, USA). This step allows better hybridisation of the cRNA to the 25-mer oligonucleotide probes. Controls (Eukaryotic Hybridisation mix, bovine serum albumin, herring sperm and B2 control oligos) and hybridisation buffer were added and the mix was denatured and pipetted into the pre-incubated Barley1 GeneChip. Incubation of the chips was made at 45°C in a rotating hybridisation oven for 16 hrs. After hybridisation, the chips were washed with stringent and non stringent buffers and stained with streptavidin using Fluidics Stations 450 (Affymetrix) The Genechips were subsequently scanned. All the protocols were recommended by Affymetrix and the Functional Genomic Centre of Zürich and can be also seen in the barley database http://barleybase.org/ (Close et al., 2004). To test the reproducibility of the technique, three replicate samples for each growth conditions were hybridised on the Barley1 Affymetrix chips.

4.2.3 Normalisation and data analysis

Expression data of the 11 pairs of each probe set were analysed with Microarray Suite Software (MAS) 5.0 and dChip package (Li and Wong, 2003). These two programmes are commonly used to analyse GeneChip data and are based on two different methods. The MAS 5.0 software from Affymetrix is a non-parametric statistical method whereas dChip uses an intensity-modelling approach (Rajagopalan, 2003). The software dChip is considered more robust compared to MAS 5.0 as it removes outlier probe intensities and reduces variability among the replicates (Irizarry et al., 2003; Barash et al., 2004). Data were normalised between the six chips with the dChip software using the baseline array with the median probe intensity values and model-based expression index. Differentially expressed genes were determined by two-class, unpaired analysis using the Excel add-in Significance of microarray Analyis (SAM (Tusher et al., 2001)), as for the cDNA microarray studies. Only genes present at least in two out of the three GeneChips for both conditions, having an absolute hybridisation intensity mean difference of 100 and detected by SAM were considered differentially expressed.

4.2.4 Phylogenetic analysis

Genes homologous to the contig3776_s_at gene, the physical impedance induced gene *IIG1* from maize and *DIR1* from Arabidopsis, all coding for putative lipid transfer protein (LTP), were identified by blasting these three sequences against the TIGR cDNA databases for Arabidopsis, rice, barley, wheat and maize. The genes of DIR1 and IIG1 have no intron and encode small proteins of 102 and 129 amino acids, respectively (Huang et al., 1998; Maldonado et al., 2002). Similarly, one open reading frame around 400bp was deduced from all the cDNA sequences. Amino acid sequences were aligned using the PILEUP program of the GCG software. Phylogenetic trees were created with the MEGA3 software package (Kumar et al., 2004) using the Dayhoff Matrix Model as amino acid substitution model to

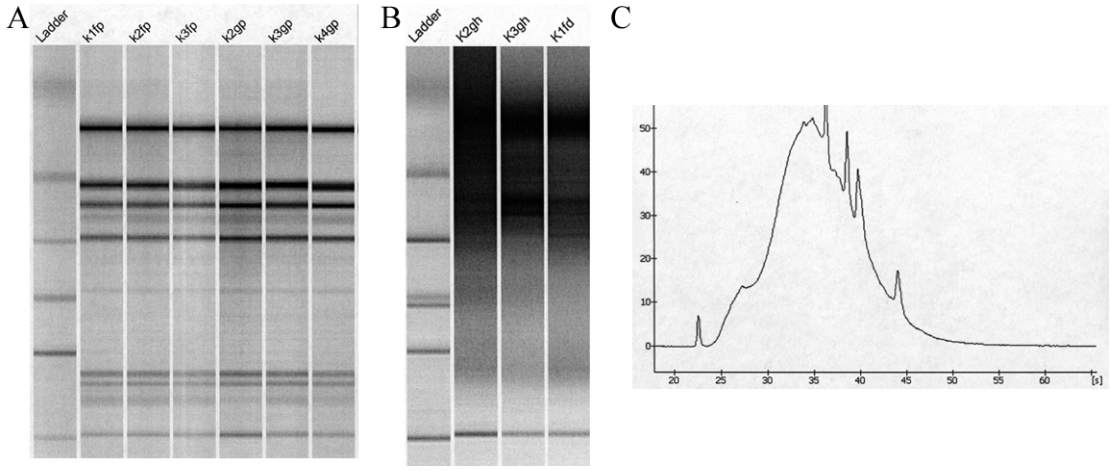


Figure 10: Preparation of the RNA and cRNA samples for GeneChip analyses using RNA Nano LabChips® on the 2100 Bioanalyzer, Agilent Technologies. A: Quality of the six samples of total RNA displayed as a gel-like electrophoregram. k1fp-k3fp: field samples; k2gp-k4gp: greenhouse samples. B: Representations as a gel-like electrophoregram of migration pattern of cRNA samples of the samples K2gh and K3gh from the greenhouse and K1fd from the field. Smears are representative of cRNA preparation of good quality. C: Signal integration of the electrophoregram for one cRNA sample showing the large pick of a successful preparation.

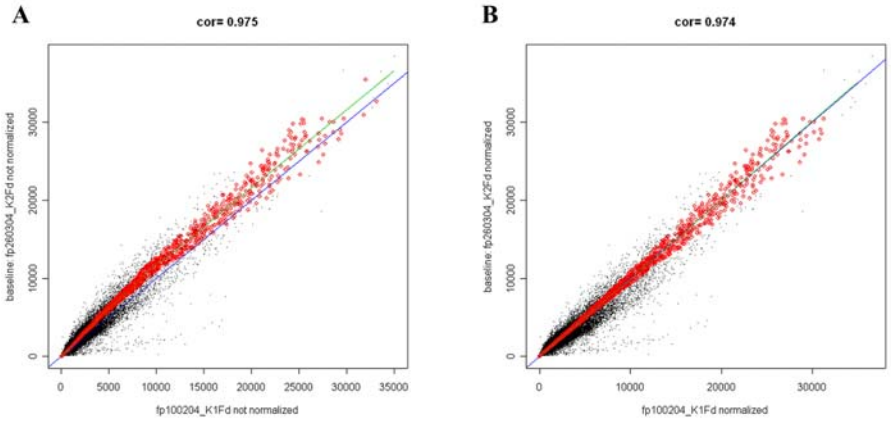


Figure 11: Scatter plots illustrating the 22,841 genes expression between the two field replicates K1 and K2. A: Before normalisation. B: After normalisation using the software dChip. The blue line is the function y=x, the red circles are the probes selected for normalisation and qualified as invariant by the software dChip. The green curve corresponds to the median based on the probes used for normalisation. The deviation of the blue line and the green curve indicates the need for normalization (that is, one array is brighter than the other). The curves were superimposed after normalisation of the data sets.

calculate evolutionary distance, and the Minimum Evolution and Pairwise Deletion methods to draw the tree (Nei and Kumar, 2000).

4.3 Results and discussion

4.3.1 Hybridisation using the GeneChip microarray

Successful use of a microarray platform from a specific grass species with probes derived from another grass species has been obtained with a cDNA microarray (Negishi et al., 2002) and with the oligonucleotide microarray from Affymetrix (Barley1 GeneChip (Close et al., 2004)). This microarray platform covers 22,792 barley genes, 21,439 probe sets of which are non-redundant sequences. Homologous hybridisations have given between 44% and 58% of present (P) calls (i.e. showing sufficient hybridisation intensity) depending on the treatment or developmental stage using the MAS 5.0 software. Cross hybridisation with different cereal species at the seedling stage resulted in 5.6% for maize up to 23.6% for wheat of P calls (Close et al., 2004). After successful preparation of our probes (Figure 10) and hybridisation on the Barley1 GeneChips, MAS5.0 gave between 29.2 to 33.8% of present probes for greenhouse grown samples and between 32.1 and 35.2% for the field grown samples. However, using the software dChip for normalisation of the chips (Li and Wong, 2003), this percentage reached between 42.7 and 45.9% of P genes for the greenhouse samples and between 43.9 and 47.8% for the field samples (around 10,300 genes for all chips). A normalisation result example is shown in Figure 11 where two replicate sets of data were plotted against each other showing a good reproducibility of the hybridisations. Because of its better high-level analysis procedures for probe selection preventing cross-hybridisation errors and contamination, the more robust software dChip was used for all the following analyses (Li and Wong, 2003; Wang et al., 2004). Only 2.4% of the probes gave marginal results (probes showing less specificity than present calls but more than absent calls) with MAS 5.0

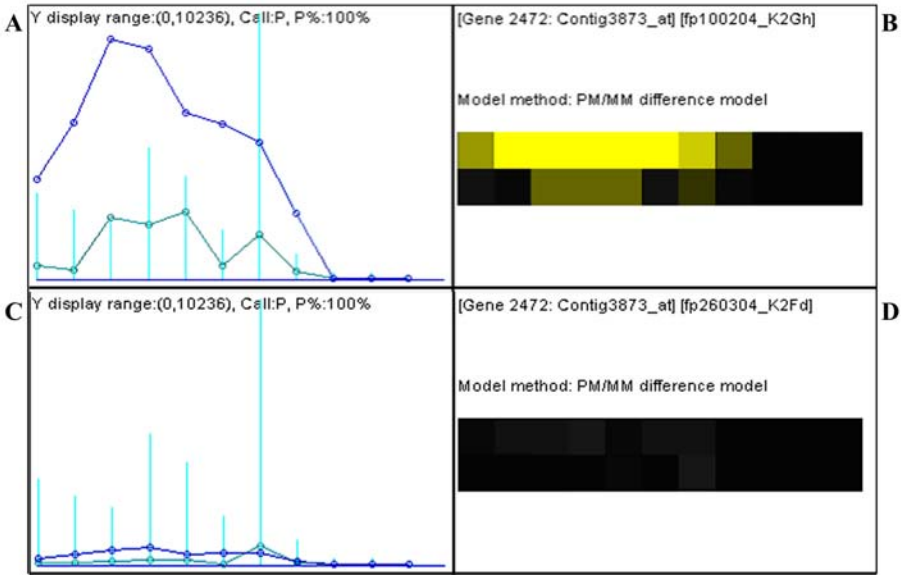


Figure 12: Hybridisation representation of a probe set (contig3873_at, coding for the LHY protein) from the Barley 1 GeneChip from Affymetrix using the software dChip. A and B: hybridisation result for the greenhouse sample, K2Gh; C and D: hybridisation result for the field sample, K2Fd. A and C: the curves represent the integration signal for each probe of the probe set with the perfect match (PM) data in dark blue and the mismatch (MM) in light blue. B and D: intensity images for the 11 probes of the probe set with the PM data in the squares of the top line and the MM in the squares of down line.

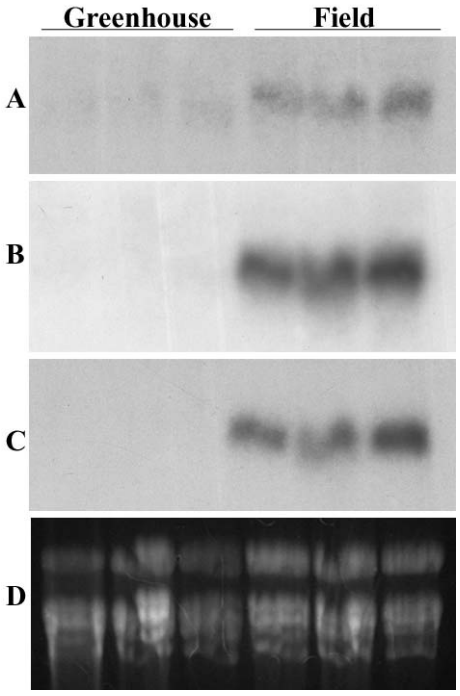


Figure 13: Northern blot analysis showing differential expression between the field- and greenhouse-grown samples for the chitinase gene (HV_CEb0003A01f, A), PR1a gene (HV_CEb0006J08f, B) and WIR 1a gene (C). Representation of the ethidium bromide-stained total RNA gel is shown in D as RNA loading control.

and less than 0.5% with dChip, meaning that probe specificity was sufficient to prevent false positive detection due to the cross-hybridisation between the wheat cRNA and the barley-derived oligonucleotides and the possible sequence differences between the two species. These results indicate that cross-hybridisation between wheat samples and the barley GeneChip was sufficient to be successfully used for large scale wheat gene expression analyses. In Figure 12, an example of hybridisation is shown for the contig3873_at probe set, representing a gene coding for the LHY protein and showing higher expression in the greenhouse compared to the field.

4.3.2 Comparison between growth conditions of wheat using cDNA microarray

In the cDNA microarray experiment, 19 genes were higher expressed in the field than in the greenhouse (Table 5). 17 of these genes belonged to the defence-response family. Three pathogenesis-related protein genes, the thaumatin-like WIR232, the WIR1c and WCI5 genes were between three to nine times up-regulated. Four chitinase and two β-1,3-endoglucanase genes as well as two peroxidase and the glutathione peroxidase-like genes were up to 6.4-fold induced. These results were confirmed for the PR 1a (HV_CEb0006J08f), chitinase (HV_CEb0003A01f), and WIR1a genes by Northern blot analysis (Figure 13). A kinase gene and an endoplasmin (HSP90) gene also showed around three-fold higher expression level compared to the greenhouse grown plants. In contrast, only two genes were higher expressed in the greenhouse than in the field (Table 5). The RNAse S-like protein precursor gene was more than eight-fold higher expressed in controlled conditions than in the field (this result is discussed in the last paragraph) and the inositol-3-phosphate synthase gene nearly three-fold.

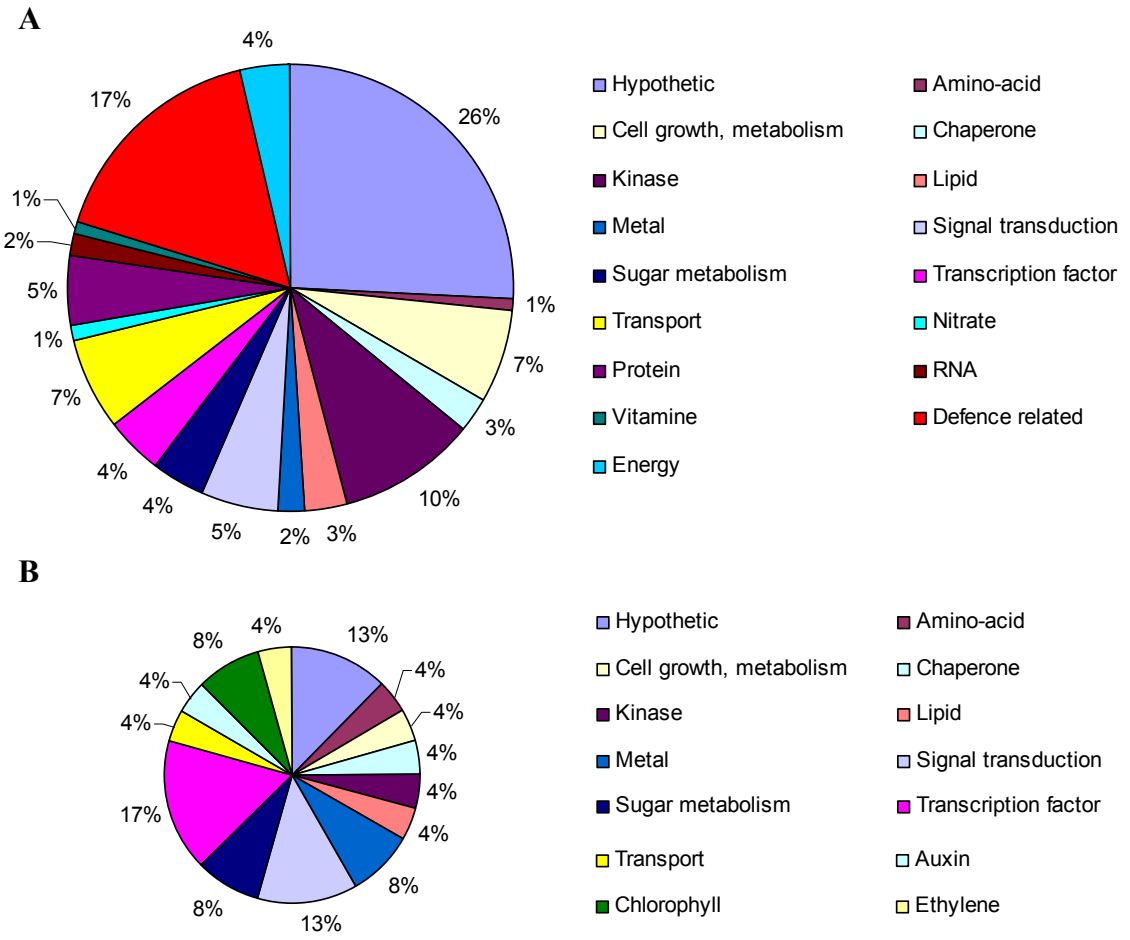


Figure 14: Pie charts representing gene family proportions of the 280 genes differentially expressed between the field and greenhouse using the Barley1 GeneChip from Affymetrix. A: Over-expressed genes (256) in the field. B: Higher expressed genes (24) in the greenhouse.

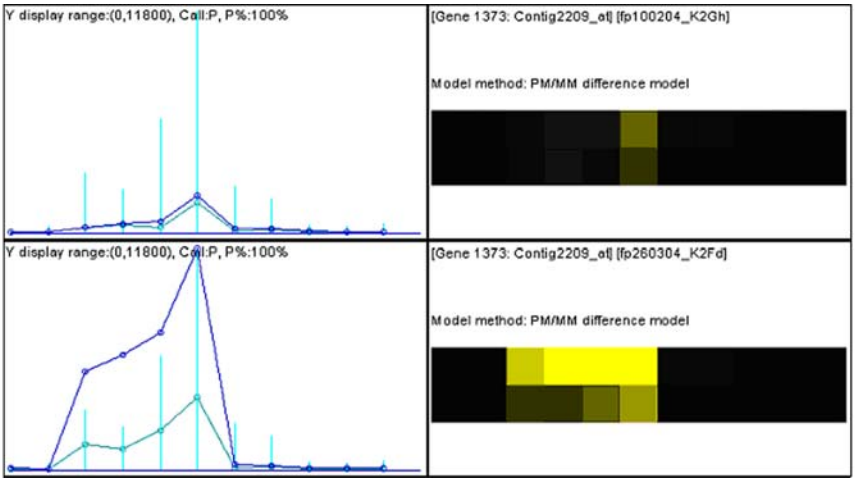


Figure 15: Hybridisation result, as in Figure 12, of the probe set contig2209_at, representing the pathogenesis-related 1a gene. Bright signals were visible for four probes out the 11 for the field sample (K2Fd, bottom) and only a weak non specific hybridisation signal was visible for one of the 11 probes for the greenhouse sample (K2Gh, top).

4.3.3 GeneChip microarray analysis: Genes with higher expression in the field

In order to extend the results obtained with our cDNA chip containing 600 genes, hybridisations were made with the GeneChip and similar patterns of gene expression as with the cDNA microarray were obtained for the defence-related gene family (Table 6). In total, 256 genes were higher expressed in the field than in the greenhouse. 26% of these genes were coding for hypothetical proteins (Figure 14).

As found with the cDNA microarrays, more genes of the chip were induced under field growth conditions than in the greenhouse. The defence-related genes represented the major class of induced genes under field conditions (Figure 14A, Table 6). Two 1,3-endoglucanase genes and a chitinase gene showed similar 5.5 to 8.5-fold induction patterns as in the cDNA microarray experiment (probes HV_CEb0010G19f, gluc2 and HV_CEb0003A01f, respectively). This result was also confirmed by Northern blot analysis for the HV_CEb0003A01f probe (Figure 13A). Similar ratios as with cDNA microarray analyses were generally obtained except for a PR1 gene (Contig2209_at) which was 66-fold induced instead of only 4.5-fold in cDNA microarrays.

Table 5: Differentially expressed genes in the field (FD) compared to greenhouse (GH) conditions determined by cDNA microarray analysis. Intensity means for the three replicates for each condition, corresponding standard deviations (sd) and fold change ratio between the two means are indicated. The positive values indicate higher gene expression in the field and negative values higher gene expression in the greenhouse.

Category	Gene ID	Accession number	Putative function	GH mean	GH mean'sd	FD mean	FD mean'sd	**fold change**
Amino-acid	HVSMEh0088J22f	BE195219	Alanine Aminotransferase 2 (Glutamic-Alanine Transaminase 2) (Alaat-2) (EC 2.6.1.2)	3098.8	308.1	6356.5	1644.9	**2.1**
Defence related	HV_CEb0010L20f	BE216529	UP|PR1_HORVU (Q05968) Pathogenesis-related protein 1 precursor	2484.4	1574.7	22153.7	6760.8	**8.9**
	wir232	TATHAU	UP|Q94F70 (Q94F70) Thaumatin-like protein	3434.1	2006.0	30388.4	8307.8	**8.8**
	HV_CEb0006J08f	BE215358	UP|PR1A_HORVU (P32937) Pathogenesis-related protein 1A/1B precursor	2065.8	1348.7	17822.9	7967.8	**8.6**
	wir1c	TARNAWIR1	UP|Q41581 (Q41581) WIR1 protein	3712.6	130.5	24452.5	4743.8	**6.6**
	HV_CEb0009I06f	BE216122	similar to UP|Q9XEN6 (Q9XEN6) Chitinase IV	7871.2	3881.4	50100.6	25379.5	**6.4**
	HV_CEb0003A01f	BE214283	UP|Q43764 (Q43764) Chitinase (EC 3.2.1.14)	1143.5	218.4	6669.8	2214.6	**5.8**
	gluc2	TAY18212	UP|Q9XEN5 (Q9XEN5) Beta-1 3-glucanase	4575.0	911.8	25930.0	8092.8	**5.7**
	HV_CEb0024H14f	BE559397	UP|PR12_HORVU (P35792) Pathogenesis-related protein PRB1-2 precursor	9725.5	1273.4	45018.4	14548.7	**4.6**
	HV_CEb0010G19f	BE216411	UP|E13B_HORVU (P15737) Glucan endo-1 3-beta-glucosidase GII precursor ((1->3)	7992.4	3634.7	32530.6	12346.3	**4.1**
	HV_CEb0009D03f	BE216036	similar to UP|Q9XEN6 (Q9XEN6) Chitinase IV	15391.1	4700.4	56046.4	19412.4	**3.6**
	wci5	TAU32431	PIR|T06278 benzothiadiazole-induced protein (clone WCI-5) - wheat {Triticum aestivum;}	8914.6	120.6	27722.4	5809.7	**3.1**
	HV_CEb0002C16f	BE214080	UP|ENPL_HORVU (P36183) Endoplasmin homolog precursor (GRP94 homolog)	15346.3	2667.2	46957.3	10264.9	**3.1**
	HV_CEb0024H02f	BE559387	UP|Q43764 (Q43764) Chitinase (EC 3.2.1.14)	3839.6	775.1	11571.6	2778.8	**3.0**
	pox381	X56011	UP|Q43212 (Q43212) Peroxidase precursor (EC 1.11.1.7)	1598.4	685.5	4789.9	1262.0	**3.0**
	HV_CEb0021P01f	BE519980	UP|Q40068 (Q40068) Peroxidase (EC 1.11.1.7)	1212.8	295.9	2992.2	447.6	**2.5**
	SFR006.D07F990622	BE437684	Thiol Protease Aleurain Precursor Gi|19021|Emb|CAA28804.1| (X05167)[Hordeum vulgare]	3356.0	434.8	7498.1	1041.6	**2.2**
	HV_CEb0016N18f	BE519542	UP|Q9SME4 (Q9SME4) Glutathione peroxidase-like protein GPX54Hv	2836.8	938.6	6176.1	1855.6	**2.2**
Kinase	SFR001.D01F990616	BE437324	Probable Serine/Threonine-Specific Protein Kinase (EC 2.7.1.-) F21P8.170 – Arabidopsis thaliana	2749.2	465.4	9691.2	2339.2	**3.5**
Signal transduction	HV_CEa0016M13f	BF267043	UP|Q9M6N6 (Q9M6N6) RNase S-like protein	54296.0	21154.4	6228.7	717.2	**-8.3**
Inositol	HVSMEg0006G05f	BG343558	UP|INO1_HORVU (O65195) Inositol-3-phosphate synthase	17569.8	4630.3	5971.1	821.2	**-2.9**

This difference between the two techniques could be due to a higher specificity of the oligonucleotide chip for the corresponding probe set (Figure 15). Transcript amounts of five genes related to disease resistance, such as the Mlo8 and Yr10 type of genes, and receptor-like kinase genes, such as LRK1, 10 and Xa21 genes, were enhanced between two and six-fold. Five glutathione-S-transferase genes, involved in detoxification processes, and eight other stress responsive genes (peroxidase and proteinase genes) were also up-regulated compared to greenhouse conditions (Table 6). The gene showing the highest induction of expression compared to the greenhouse was a lipid transfer protein gene, belonging to the PR14 family (Mills et al., 2004), with a 92-fold induction factor (Figure 16, Table 6). The sequence of this gene is highly similar to the sequence of the physical impedance induced protein gene *IIG1* from *Zea mays* (Huang et al., 1998) which is proposed to be one of the early components of the stress signal transduction pathway. Another lipid transfer protein, DIR1 in Arabidopsis, is considered to be a candidate for the long distance signal for systemic resistance (Maldonado et al., 2002). A recent study has shown that LTP1 from tobacco binds jasmonic acid with high affinity (Buhot et al., 2004). The resulting complex is recognised by the elicitin receptor and long distance protection against fungi attack is induced by local application of this complex (Buhot et al., 2004). However, there is no evidence that this complex is the mobile signal. The high over-expression ratio of the putative LTP gene in the field could imply that its corresponding protein has a similar function in wheat as in the dicots for signalling in defence response.

Interestingly, eleven genes coding for transcription factors, ten for ribosomal proteins, three for proteins involved in protein maturation, 25 for kinases and 14 for proteins involved in signal transduction were also significantly over-expressed in the field (Table 6). Some of these genes are probably involved in the triggering and establishment of the defence response. Furthermore, eight chaperone genes were induced between four and 7.5-fold, and four genes

Table 6: Highly expressed genes in the field (Fd) compared to greenhouse (Gh) conditions determined by GeneChip analysis. Signal intensity means for the three replicates for each condition and corresponding standard deviation (sd) and fold change ratio between the two means are indicated.

Category	Probe ID	Best match accession number and putative fonction	Gh mean	Gh mean's sd	Fd mean	Fd mean's sd	**fold change**
Amino acid	HV12E23u_at	AAD23908.1 1e-45 (AF073696) cysteine synthase [Oryza sativa]	63.49	15.08	184.54	19.05	**2.9**
	Contig15840_at	NP_568496.1 4e-38 (NM_122623) putative amino acid aminotransferase	220.89	22.83	481.15	24.87	**2.2**
Cell growth, division	Contig1393_at	T51179 e-105 actin [imported] - garden pea gb|AAB18641.1	760.26	42.85	1575.26	68.31	**2.1**
Cell wall	Contig10022_at	BAC21356.1 2e-05 (AP003816) putative glycine-rich cell wall protein precursor [Oryza sativa (japonica cultivar-group)]	424.76	54.68	1432.71	29.53	**3.4**
	Contig631_at	AAF70818.1 1e-97 root cap-specific protein [Zea mays] GDP-mannose 4.6-dehydratase activity	243.30	23.02	737.69	53.13	**3.0**
	Contig2672_at	T02090 2e-64 xyloglucan endo-1.4-beta-D-glucanase (EC 3.2.1.-) - maize	524.79	72.07	1300.33	34.72	**2.5**
	Contig1642_at	CAA77237.1 e-146 reversibly glycosylated polypeptide [Triticum aestivum]	851.67	45.12	2056.98	42.65	**2.4**
	Contig306_s_at	gb|AAQ24632.1 3e-067 GPRP [Oryza sativa (indica cultivar-group)]	1281.60	106.67	3049.08	50.24	**2.4**
Chaperone	Contig2720_at	T06489 e-110 probable peptidylprolyl isomerase (EC 5.2.1.8) FKBP77 - wheat	47.73	8.10	358.51	18.59	**7.5**
	rbags16i08_s_at	BAC06263.1 7e-11 putative calreticulin [Oryza sativa (japonica cultivar-group)]	107.53	8.74	496.89	18.17	**4.6**
	L32165_s_at	T05741 0.0 dnaK-type molecular chaperone HSP70 - barley gb|AAA62325.1| HSP70	632.00	124.89	2829.79	58.63	**4.5**
	Contig1615_s_at	T03581 8e-94 dnaK-type molecular chaperone BiP - rice gb|AAB63469.1|	686.21	121.06	2763.16	163.72	**4.0**
	Contig71_s_at	P36183 2e-50 endoplasmin homolog precursor (GRP94 HOMOLOG)	673.06	58.81	2665.67	109.15	**4.0**
	Contig2747_at	BAC06263.1 e-114 putative calreticulin [Oryza sativa (japonica cultivar-group)]	651.04	157.69	2537.84	49.93	**3.9**
	Contig2747_x_at	BAC06263.1 e-114 putative calreticulin [Oryza sativa (japonica cultivar-group)]	631.77	125.90	2361.32	103.60	**3.7**
Defence related	Contig2209_at	S37166 5e-81 pathogenesis-related protein 1a - barley emb|CAA52893.1|	28.22	13.17	1864.03	155.52	**66.1**
	Contig19003_at	BAB89090.1 3e-056 (AP003372) similar to acetone-cyanohydrin lyase [Oryza sativa (japonica cultivar-group)]	29.78	8.60	275.91	16.21	**9.3**
	Contig1636_at	Q02126 e-164 glucan endo-1.3-beta-glucosidase GIII precursor ((1->3)	22.36	0.00	189.42	17.61	**8.5**
	Contig4326_s_at	AAD28733.1 4e-69 chitinase IV precursor [Triticum aestivum]	564.82	128.14	4201.64	197.59	**7.4**
	Contig16386_at	AAG21897.1 7e-68 putative disease resistance protein (3 partial) [Oryza sativa]	422.33	35.05	2577.43	70.92	**6.1**
	Contig19684_at	AAG13627.1 5e-35 (AC078840) putative hypersensitivity-related (hsr)protein [Oryza sativa (japonica cultivar-group)]	381.11	69.81	2272.82	90.72	**6.0**
	Contig1639_at	S35156 e-106 beta-glucanase - barley	395.18	45.11	2150.20	47.56	**5.4**
	Contig86_at	P05167 e-129 Thiol protease aleurain precursor emb|CAA28804.1| [Hordeum vulgare]	426.03	175.67	1821.33	60.29	**4.3**
	Contig3884_at	AAD04231.1 1e-089 PDI-like protein [Zea mays]	515.46	59.62	2172.45	57.36	**4.2**
	Contig2946_at	BAA77282.1 2e-97 monodehydroascorbate reductase [Oryza sativa (japonica cultivar-group)]	250.80	48.41	1055.69	73.83	**4.2**
	HT08H03u_s_at	CAA72790.1 7e-34 cysteine proteinase inhibitor [Hordeum vulgare subsp. vulgare]	476.94	83.20	2008.79	99.28	**4.2**
	Contig3563_at	dbj|BAC22233.1 2e-059 ethylene-forming-enzyme-like dioxygenase-like protein [Oryza sativa (japonica cultivar-group)]	75.41	10.09	314.74	39.35	**4.2**

Category	Probe ID	Best match accession number and putative fonction	Gh mean	Gh mean's sd	Fd mean	Fd mean's sd	**fold change**
Defence related	Contig9355_s_at	dbj|BAB64773.1 4e-071 P0583G08.8 [Oryza sativa (japonica cultivar-group)]	145.18	29.79	572.09	47.54	**3.9**
	Contig2680_at	T06413 e-119 cathepsin B-like cysteine proteinase (EC 3.4.22.-) - wheat (fragment)	524.75	97.85	2034.35	27.77	**3.9**
	Contig18367_at	AAM12330.1 6e-054 (AC091680) putative glutathione S-transferase [Oryza sativa (japonica cultivar-group)]	87.11	16.16	336.22	45.72	**3.9**
	Contig1728_at	CAA06996.1 e-142 ascorbate peroxidase [Hordeum vulgare subsp. vulgare]	1352.72	128.89	4937.90	150.21	**3.7**
	Contig6505_at	dbj|BAB03288.1 2e-023 hydrophobic polypeptide [Triticum aestivum] Length = 54; 2. gb|AAA21847.1 2e-023 salt-stress induced hydrophobic peptide [Lophopyrum elongatum]	67.37	14.89	231.19	20.69	**3.4**
	Contig5943_s_at	T50649 1e-63 elicitor-responsive gene 3 [imported] - rice gb|AAC35866.1|	397.26	92.31	1359.87	67.85	**3.4**
	Contig725_s_at	AAA70346.1 e-151 disulfide isomerase	387.65	33.82	1313.71	35.90	**3.4**
	Contig16273_at	AAK38344.1 3e-95 (AY029319) seven transmembrane protein Mlo8 [Zea mays]	191.49	30.16	644.49	21.37	**3.4**
	baak13l10_s_at	AAM94294.1 6e-05 putative stripe rust resistance protein Yr10 [Sorghum bicolor]	162.21	39.81	540.48	23.57	**3.3**
	Contig14242_at	BAB68104.1 2e-41 (AP003734) putative cinnamoyl CoA reductase [Oryza sativa (japonica cultivar-group)]	202.98	26.41	645.36	29.56	**3.2**
	Contig1737_at	T05943 e-141 probable lipoxygenase (EC 1.13.11.12) - barley gb|AAB60715.1|	96.27	24.95	289.19	11.53	**3.0**
	Contig4298_at	BAB18768.1 3e-60 cysteine proteinase inhibitor [Triticum aestivum]	1232.36	300.74	3674.36	61.40	**3.0**
	Contig13516_at	BAB89968.1 3e-57 putative Cf2/Cf5 disease resistance protein [Oryza sativa (japonica cultivar-group)]	88.91	18.17	263.96	22.84	**3.0**
	Contig2932_at	dbj|BAB17730.1| putative leucine-rich repeat protein LRP [Oryza sativa]	901.88	165.57	2663.75	110.93	**3.0**
	Contig2442_at	NP_171990.2 4e-82 disulfide isomerase-related protein. putative [Arabidopsis]	213.98	29.23	601.22	16.18	**2.8**
	Contig4044_at	AAK38509.1 3e-68 (AC087181) putative glutathione S-transferase [Oryza sativa]	1102.28	178.12	2978.58	181.60	**2.7**
	Contig3054_s_at	AAC34855.1 4e-66 senescence-associated protein 5 [Hemerocallis hybrid cultivar]	953.06	85.85	2469.57	39.19	**2.6**
	Contig3801_at	AAN17462.1 3e-72 hypersensitive-induced reaction protein 1 [Hordeum vulgare]	91.99	6.92	236.74	29.01	**2.6**
	Contig2453_at	CAB59895.1 8e-92 glutathione peroxidase-like protein GPX54Hv [Oryza sativa (japonica cultivar-group)]	866.73	54.81	2212.58	96.47	**2.6**
	Contig23747_at	BAB89560.1 2e-07 (AP003251) putative Avr9/Cf-9 rapidly elicited protein	211.76	27.85	537.44	35.20	**2.5**
	Contig4185_at	BAB16331.1 2e-86 putative r40c1 protein [Oryza sativa (japonica cultivar-group)]	765.93	67.49	1933.45	50.65	**2.5**
	Contig2425_s_at	CAD11966.1 e-119 glutathione-S-transferase. I subunit [Hordeum vulgare subsp. vulgare]	706.78	113.24	1777.77	47.97	**2.5**
	Contig3800_s_at	AAN17462.1 e-126 hypersensitive-induced reaction protein 1 [Hordeum vulgare]	1483.34	123.09	3619.75	92.34	**2.4**
	HVSMEh0083O16r2_s_at	CAD11966.1 2e-34 glutathione-S-transferase. I subunit [Hordeum vulgare subsp. vulgare]	659.88	77.43	1605.69	30.84	**2.4**
	rbags13d01_s_at	AAB99745.1. HSP70 [Triticum aestivum] . Expect:0.	1659.17	182.42	3891.17	165.34	**2.4**
	HVSMEn0023O21f_s_at	BAA88898.1 8e-08 cysteine protease component of protease-inhibitor complex [Zea mays]	936.38	114.89	2204.34	117.44	**2.4**
	Contig919_s_at	NP_909921.1. putative thioredoxin [Oryza sativa (japonica cultivar-group)] gb|AAO37523.1|	547.36	49.77	1209.09	60.33	**2.2**
	Contig1727_s_at	AAL08496.1 e-123 ascorbate peroxidase [Hordeum vulgare]	3208.66	191.79	6969.42	251.07	**2.2**
	Contig7171_s_at	AAL47687.1 8e-70 glutathione-S-transferase Cla47 [Triticum aestivum]	288.33	15.51	601.54	36.68	**2.1**
	Contig4803_at	Q84LL6, Salt tolerance protein 5	610.92	35.90	1214.46	36.88	**2.0**
	Contig2402_s_at	Q10716 e-108 Cysteine proteinase 1 precursor pir||S59597	2952.85	188.09	5755.39	103.09	**2.0**

Category	Probe ID	Best match accession number and putative fonction	Gh mean	Gh mean's sd	Fd mean	Fd mean's sd	**fold change**
Energy	Contig5922_at	AAK54299.1 2e-99 putative thiolase [Oryza sativa (japonica cultivar-group)]	327.10	26.99	1550.29	44.65	**4.7**
	Contig14714_at	CAC40028.1 e-116 (AJ310840) P-type ATPase [Hordeum vulgare]	347.90	66.26	1301.14	75.99	**3.7**
	Contig5888_at	BAB88645.1 e-125 (AB078882) alternative oxidase [Triticum aestivum]	183.76	18.92	610.94	11.70	**3.3**
	Contig431_at	AAK49116.1 e-111 alcohol dehydrogenase [Hordeum vulgare]	66.76	15.86	214.99	20.73	**3.2**
	HI04E24r_s_at	BAB90185.1 2e-72 (AP003407) putative allyl alcohol dehydrogenase [Oryza sativa (japonica cultivar-group)]	182.95	28.58	473.15	30.48	**2.6**
	Contig3477_at	NP_201477.1 e-126 succinate dehydrogenase flavoprotein alpha subunit (emb|CAA05025.1);	248.46	35.25	640.67	24.55	**2.6**
	Contig4530_at	BAA82749.1 e-131 (AB017428) succinate dehydrogenase iron-protein subunit (SDHB) [Oryza sativa (japonica cultivar-group)]	404.29	23.27	884.29	31.24	**2.2**
	Contig840_s_at	JC1466 7e-73 inorganic diphosphatase (EC 3.6.1.1) - barley	704.53	63.50	1463.52	75.82	**2.1**
	Contig11449_at	CAC40031.1 e-103 P-type ATPase [Hordeum vulgare]	327.10	26.99	1550.29	44.65	**1.9**
Iron	HV12A05u_s_at	AAM74943.1 4e-34 ferritin [Oryza sativa (japonica cultivar-group)]	482.88	110.10	2292.45	65.04	**4.8**
	Contig2716_s_at	AAM74942.1 3e-79 ferritin [Oryza sativa (japonica cultivar-group)]	1197.05	87.51	4737.88	169.00	**4.0**
	Contig2715_s_at	AAM74942.1 1e-93 ferritin [Oryza sativa (japonica cultivar-group)]	123.00	37.13	461.95	18.29	**3.8**
Kinase	Contig14572_at	AAK02024.2 7e-86 (AC074283) Putative protein kinase [Oryza sativa]	28.49	5.70	287.99	32.01	**10.1**
	AF085166_at	AAD44031.1 0.0 receptor-like kinase [Hordeum vulgare]	153.82	58.83	1150.86	73.23	**7.5**
	AF085166_x_at	AAD44031.1 0.0 receptor-like kinase [Hordeum vulgare]	155.13	54.21	1128.70	39.90	**7.3**
	Contig14031_at	BAB40022.1 1e-81 (AP003021) putative wall-associated kinase 1 [Oryza sativa (japonica cultivar-group)]	52.94	7.71	372.19	25.21	**7.0**
	Contig12770_at	BAA95893.1 7e-88 Similar to Arabidopsis thaliana wak4 gene; wall-associated kinase 4. (AJ009695) [Oryza sativa (japonica cultivar-group)]	179.34	45.12	939.39	22.37	**5.2**
	Contig21059_at	BAC06926.1 1e-19 (AP003758) putative receptor-type protein kinase LRK1 [Oryza sativa (japonica cultivar-group)]	170.35	35.99	887.63	19.35	**5.2**
	Contig13693_at	AAK20737.1 9e-55 TAK19-1 [Triticum aestivum]	313.77	25.32	1604.68	44.27	**5.1**
	Contig13693_x_at	AAK20737.1 9e-55 TAK19-1 [Triticum aestivum]	320.90	30.65	1619.25	83.29	**5.1**
	Contig11886_s_at	emb|CAD40528.1 2e-072 OSJNBa0023J03.16 [Oryza sativa (japonica cultivar-group	149.57	27.25	736.79	40.40	**4.9**
	rbaal11f18_at	BAC20671.1 7e-56 serine/threonine kinase-like protein [Oryza sativa (japonica cultivar-group)]	51.59	8.80	242.47	14.85	**4.7**
	Contig13692_x_at	gb|AAK20744.1 0 TAK14 [Triticum aestivum] Length = 689; 2. gb|AAD44032.1 0 AF085167_1 receptor-like kinase ARK1AS [Hordeum vulgare]	261.21	59.79	1073.03	29.39	**4.1**
	Contig7366_at	T06793 e-112 receptor kinase homolog LRK10 - wheat gb|AAC49629.1| (U51330)	76.12	19.29	246.97	9.44	**3.2**
	Contig24882_at	AAL25569.1 3e-15 (AY058153) At2g31880/F20M17.8 [Arabidopsis thaliana]	1479.83	210.30	4712.62	83.48	**3.2**
	Contig7260_at	gb|AAP55049.1 0 putative casein kinase II beta subunit [Oryza sativa (japonica cultivar-group)]	182.27	42.89	561.10	38.01	**3.1**
	Contig6958_s_at	BAC20671.1 7e-83 serine/threonine kinase-like protein [Oryza sativa (japonica cultivar-group)]	698.92	142.40	2114.37	58.71	**3.0**
	EBro08_SQ012_G10_at	BAB92400.1 1e-06 (AP003276) putative protein kinase Xa21 [Oryza sativa (japonica cultivar-group)]	58.94	16.07	175.53	8.27	**3.0**
	Contig4998_s_at	AAK20741.1 1e-82 TAK33 [Triticum aestivum]	259.95	21.97	666.93	21.42	**2.6**

Category	Probe ID	Best match accession number and putative fonction	Gh mean	Gh mean's sd	Fd mean	Fd mean's sd	**fold change**
Kinase	Contig5531_at	S56638 e-127 mitogen-activated protein kinase 1 homolog (clone Aspk9) -[Avena sativa]	295.47	34.89	737.74	24.98	**2.5**
	Contig24385_at	BAC10827.1 1e-75 putative protein kinase Xa21. receptor type precursor [Oryza sativa (japonica cultivar-group)]	1981.82	214.19	4848.79	125.60	**2.5**
	Contig6447_at	P28583 e-108 Calcium-dependent protein kinase SK5 (CDPK) pir||A43713 soybean	259.34	21.22	633.48	42.35	**2.4**
	Contig4696_at	AAF23371.1 3e-93 (AF187062) UMP/CMP kinase a [Oryza sativa]	776.45	97.43	1890.84	118.14	**2.4**
	Contig6203_at	BAC10827.1 3e-50 putative protein kinase Xa21. receptor type precursor [Oryza sativa (japonica cultivar-group)]	109.42	11.97	264.31	22.19	**2.4**
	Contig10249_at	NP_568696.1 8e-46 receptor-like protein kinase; protein id: At5g48380.1	132.77	14.67	315.79	16.78	**2.4**
	Contig15860_at	NP_174702.1. leucine-rich repeat family protein / protein kinase family protein [Arabidopsis thaliana]	236.40	25.45	522.94	15.72	**2.2**
	Contig4996_at	AAK20741.1 2e-79 TAK33 [Triticum aestivum]	326.45	37.56	693.52	39.33	**2.1**
Lipid	Contig3776_s_at	AAM74427.1 2e-18 (AC123594) Putative lipid transfer protein [Oryza sativa (japonica cultivar-group)]	32.57	23.13	2998.92	155.97	**92.1**
	Contig9086_at	BAB90753.1 e-103 putative serine palmitoyltransferase [Oryza sativa (japonica cultivar-group)]	317.57	86.62	1280.53	64.43	**4.0**
	Contig9365_at	cytochrome b5 protein [Ricinus communis]. gb|AAP23033.1 0 sphingolipid delta-8 desaturase [Primula farinosa]	309.32	27.14	905.52	95.99	**2.9**
	Contig10724_at	AAG13623.1 1e-58 putative steroid membrane binding protein [Oryza sativa (japonica cultivar-group)]	392.94	89.24	1148.68	74.38	**2.9**
	Contig10483_at	F24J1.22 [Arabidopsis thaliana] Length = 260; 3. gb|AAL50102.1 0 At1g69640/F24J1.22	516.83	103.34	1456.17	73.95	**2.8**
	Contig23161_at	AAL73565.1 3e-64 (AC079632) Putative acyltransferase [Oryza sativa] gb|AAM08633.1|AC108883_6 (AC108883)	118.95	19.82	310.79	14.51	**2.6**
	HW08N05u_s_at	CAB61740.1 2e-07 (AJ275305) putative enoyl CoA hydratase [Cicer arietinum]	129.95	11.13	298.93	22.03	**2.3**
Membrane	Contig25699_at	NP_178286.1 3e-06 putative membrane protein; protein id: At2g01770.1 [Arabidopsis thaliana]	123.64	16.44	462.35	17.12	**3.7**
	rbags23g22_at	T03273 2e-21 embryogenesis transmembrane protein - maize emb|CAA66183.1|	70.15	23.70	225.14	17.89	**3.2**
	Contig5711_at	NP_176075.1 1e-63 integral membrane protein. putative; protein id: At1g57620.1. supported by cDNA: 17602. [Arabidopsis thaliana]	395.00	89.35	1210.38	55.62	**3.1**
	Contig5447_at	NP_568465.1 9e-96 (NM_122419) endomembrane protein 70. putative; protein id: At5g25100.1. supported by cDNA: gi_13430445 [Arabidopsis thaliana]	904.66	70.47	2098.18	102.56	**2.3**
Metal	Contig956_x_at	emb|CAD54079.1 8e-045 metallothioneine type2 [Hordeum vulgare subsp. vulgare]	1103.72	133.78	3799.55	223.05	**3.4**
	Contig6074_at	emb|CAD70173.1 1e-083 farnesylated protein 3 [Hordeum vulgare subsp. vulgare]	328.36	77.64	999.51	37.01	**3.0**
Nitrate	Contig8727_s_at	BAB63595.1 2e-71 putative formamidase [Oryza sativa (japonica cultivar-group)]	317.87	101.03	1995.92	52.75	**6.3**
	Contig5371_s_at	AAD49420.1 e-111 amine oxidase [Canavalia lineata]	368.08	53.15	1380.30	36.20	**3.8**
	Contig5370_at	AAD49420.1 2e-092 amine oxidase [Canavalia lineata]	109.13	16.32	265.23	18.09	**2.4**
Nucleotide sugar metabolism	Contig1931_at	Q8S862. Putative epimerase/dehydratase Length = 378. Expect:0. match=333/366 aa. more	415.28	58.64	1168.93	37.66	**2.8**
	Contig2918_s_at	AAL65400.1 3e-70 dTDP-glucose 4-6-dehydratase-like protein [Oryza sativa]	493.59	63.12	1188.99	60.41	**2.4**

Category	Probe ID	Best match accession number and putative fonction	Gh mean	Gh mean's sd	Fd mean	Fd mean's sd	**fold change**
Pigment	Contig8162_at	BAB92583.1 4e-93 putative 1.4-benzoquinone reductase [Oryza sativa (japonica cultivar-group)]	175.54	66.31	962.73	62.03	**5.5**
	Contig9053_at	gb|AAM64959.1 6e-092 minor allergen [Arabidopsis thaliana]	263.33	36.25	866.46	34.70	**3.3**
	Contig12883_at	dbj|BAA96953.1 0 ubiquinone/ menaquinone biosynthesis methyltransferase	97.55	15.10	305.09	31.17	**3.1**
Protein	Contig9420_at	ref|NP_568380.1 0 oligosaccharyl transferase STT3-related protein [Arabidopsis thaliana]	406.61	79.77	1479.22	110.00	**3.6**
	Contig8893_at	NP_568947.1 2e-97 signal recognition particle - like protein; protein id: At5g61970.1	131.92	34.53	450.64	21.76	**3.4**
	EBma03_SQ003_N08_s_at	NP_910672.1. contains EST AU031225(E61165)~nhp2-like protein [Oryza sativa (japonica cultivar-group)]	178.51	21.88	427.26	34.12	**2.4**
Protein synthesis	Contig2310_at	NP_187207.1 5e-41 putative 60S ribosomal protein L22; protein id: At3g05560.1	197.77	45.03	712.12	45.86	**3.6**
	Contig1950_at	Q9XHS0 5e-68 40S ribosomal protein S12 gb|AAD39838.1|AF067732_1 [Hordeum vulgare]	259.33	35.49	929.91	19.96	**3.6**
	HV_CEa0004C09r2_at	P45633 5e-34 60S ribosomal protein L10 (QM protein homolog) pir||T02068 probable transcription factor QM - maize	115.09	16.63	359.83	15.76	**3.1**
	Contig1493_at	BAB63622.1 9e-55 putative ribosomal protein S10 [Oryza sativa (japonica cultivar-group)]	130.56	18.17	378.41	23.45	**2.9**
	Contig705_at	O48558 2e-54 60S ribosomal protein L30 pir||T01411 ribosomal protein L30 - maize	454.25	92.84	1219.33	37.09	**2.7**
	Contig2609_at	NP_191308.1 e-104 40S ribosomal protein S2 homolog; protein id: At3g57490.1 [Arabidopsis thaliana]	146.87	24.44	386.07	15.98	**2.6**
	EBpi01_SQ001_K06_s_at	Q05462 6e-05 60S ribosomal protein L27 pir||T06451 ribosomal protein L27 - garden pea	585.31	85.88	1494.22	94.45	**2.6**
	Contig1753_at	P41098 1e-53 60S ribosomal protein L34 pir||S48027 ribosomal protein L34. cytosolic - common tobacco	357.41	56.12	874.64	37.65	**2.5**
	HR01C15u_s_at	BAB90499.1 2e-32 putative 60S ribosomal protein L18A [Oryza sativa (japonica cultivar-group)]	700.66	60.83	1523.48	28.01	**2.2**
	Contig870_at	O48558 5e-51 60S ribosomal protein L30 pir||T01411 ribosomal protein L30 - maize	169.27	17.24	361.19	14.33	**2.1**
RNA	Contig10436_at	CAB75505.1 9e-59 VIP1 protein [Avena fatua]	327.85	130.44	1478.19	81.99	**4.5**
	Contig8361_at	AAF40306.1 2e-56 RNA helicase [Vigna radiata]	127.16	25.33	464.95	21.61	**3.7**
	Contig742_at	T06458 6e-49 nucleolin homolog - garden pea gb|AAA74208.1|	81.55	11.48	264.64	25.20	**3.3**
	Contig471_s_at	S53050 1e-15 RNA binding protein - barley emb|CAA88558.1| glycine rich protein. RNA binding protein [Hordeum vulgare subsp.	934.52	81.77	2364.39	221.82	**2.5**
Signal transduction	Contig13201_at	NP_568919.1 2e-15 pseudo-response regulator - like; protein id: At5g60100.1 [Arabidopsis thaliana]	136.19	33.74	704.81	34.67	**5.2**
	HV_CEb0014M10r2_at	AAF89745.3 2e-15 phosphatidic acid signal transduction beta [Vigna unguiculata]	61.26	12.23	297.32	15.64	**4.9**
	Contig7098_at	dbj|BAB88216.1 0 secretory acid phosphatase precursor [Oryza sativa]	79.45	20.13	327.53	8.86	**4.1**
	Contig10995_at	JE0114 7e-75 zinc-finger protein C60910 [imported] - rice dbj|BAA33200.1| (AB001882) zinc finger protein [Oryza sativa (japonica	224.57	44.41	904.44	33.70	**4.0**
	Contig15359_at	gb|AAP13012.1 3e-072 putative calmodulin [Oryza sativa (japonica cultivar-group)]	146.45	44.69	563.29	18.89	**3.9**
	Contig1338_s_at	P13565 5e-75 calmodulin sp|P29612|CALM_ORYSA calmodulin	373.48	115.82	1415.20	73.74	**3.8**
	Contig5247_at	AAL08497.2 e-134 gigantea-like protein [Hordeum vulgare]	469.14	56.78	1690.50	63.31	**3.6**

Category	Probe ID	Best match accession number and putative fonction	Gh mean	Gh mean's sd	Fd mean	Fd mean's sd	**fold change**
Signal transduction	Contig21914_at	AAK15501.1 2e-10 (AF325719) calmodulin-like protein [Pennisetum ciliare]	105.41	28.39	335.19	20.59	**3.2**
	Contig13898_at	AAM34770.1 1e-07 nam-like protein 7 [Petunia x hybrida]	271.54	43.10	810.67	37.40	**3.0**
	Contig3857_at	NP_175669.1 9e-86 (NM_104138) signal peptidase subunit. putative; protein id: At1g52600.1. supported by cDNA: 103157	323.36	31.66	891.39	33.32	**2.8**
	Contig1330_at	AAC49582.1 3e-80 calmodulin TaCaM2-2 gb|AAC49583.1| calmodulin TaCaM2-3	1112.05	175.20	3062.90	71.79	**2.8**
	Contig12779_at	AAL86486.1 e-101 putative leucine-rich repeat protein [Oryza sativa (japonica cultivar-group)]	174.73	19.69	418.29	30.66	**2.4**
	Contig18088_at	S39045 2e-35 probable finger protein WZF1 - wheat dbj|BAA03901.1|	153.06	14.88	351.03	8.85	**2.3**
	Contig9993_at	NP_190235.1 3e-74 (NM_114518) arm repeat containing protein homolog; protein id: At3g46510.1 gi_14596006 [Arabidopsis thaliana]	311.62	23.74	662.87	31.03	**2.1**
Sugar metabolism	Contig7646_at	AAF16414.1 3e-40 (AF126550) mannosyl-oligosaccharide 1.2-alpha-mannosidase [Glycine max]	207.83	68.13	743.29	93.78	**3.6**
	Contig1019_at	AAF71272.1 e-134 ribulose bisphosphate carboxylase activase B [Triticum aestivum]	190.67	36.19	615.52	82.03	**3.2**
	Contig18334_at	NP_189051.1 4e-74 glutamine:fructose-6-phosphate amidotransferase. putative; protein id: At3g24090.1 [Arabidopsis thaliana]	149.16	30.49	437.81	34.15	**2.9**
	Contig15078_at	Q9LUG2. Alpha glucosidase-like protein	56.64	14.27	162.92	13.20	**2.9**
	Contig865_5_s_at	emb|CAD79700.1 0 putative glyceraldehyde 3-phosphate dehydrogenase [Oryza sativa (indica cultivar-group)]	991.80	86.98	2667.90	63.59	**2.7**
	Contig15045_at	T04422 1e-90 alpha-galactosidase (EC 3.2.1.22) - barley (fragment) emb|CAA74161.1|	402.93	46.36	1075.38	50.01	**2.7**
	HZ48K22r_s_at	P08477 9e-35 Glyceraldehyde 3-phosphate dehydrogenase. cytosolic	1602.43	170.25	3829.50	129.63	**2.4**
	Contig6517_at	Q9LKJ3 e-113 Alpha-glucan phosphorylase. H isozyme	1078.53	85.60	2446.03	134.22	**2.3**
	Contig2867_at	AAL99745.1 e-127 pyruvate decarboxylase [Zea mays]	249.95	26.38	525.74	15.69	**2.1**
Transcription factor	Contig8533_s_at	T03968 4e-80 probable transcription factor - rice emb|CAA71844.1| RAPB protein [Oryza sativa (indica cultivar-group)]	138.20	35.19	614.02	36.65	**4.4**
	HV_CEb0021P13r2_at	BAC07080.1 5e-20 (AP004009) similar to transcription factor MYB124 [Oryza sativa (japonica cultivar-group)]	132.67	58.62	488.44	68.42	**3.7**
	Contig3582_s_at	AAM91875.1 3e-63 putative transcription factor [Oryza sativa (japonica cultivar-group)]	116.23	36.40	422.08	26.10	**3.6**
	Contig10794_at	NP_196037.2 7e-70 EF - hand Calcium binding protein - like; protein id: At5g04170.1	459.06	47.12	1451.85	24.41	**3.2**
	Contig9574_at	AAL59028.1 e-101 (AC087182) putative HD domain protein [Oryza sativa]	72.69	19.01	221.79	21.72	**3.1**
	Contig18080_at	BAB63582.1 2e-53 P0403C05.2 [Oryza sativa (japonica cultivar-group)]	278.68	18.08	776.22	33.67	**2.8**
	Contig6159_at	NP_180003.1 7e-35 bHLH protein; protein id: At2g24260.1. supported by cDNA: gi_20127071 [Arabidopsis thaliana]	93.84	9.44	261.51	14.37	**2.8**
	rbaak22p05_s_at	NP_191427.1 7e-25 transcriptional coactivator - like protein; protein id: At3g58680.1	1485.32	248.08	3857.61	51.41	**2.6**
	Contig7481_at	BAB84620.1 9e-24 (AP003450) DNA-binding protein RAV2-like [Oryza sativa (japonica cultivar-group)]	66.09	21.81	168.36	13.48	**2.6**
	Contig3958_s_at	gb|AAP15161.1 0 superal1 [Zea mays]	603.45	66.98	1472.49	55.53	**2.4**
	Contig995_at	Q40034 e-116 Elongation factor 1-alpha (EF-1-alpha) pir||S39505 - barley	396.23	26.49	790.96	35.07	**2.0**

Category	Probe ID	Best match accession number and putative fonction	Gh mean	Gh mean's sd	Fd mean	Fd mean's sd	**fold change**
Transport	Contig6707_at	AAL14615.1 6e-95 putative sugar transporter [Oryza sativa]	122.84	25.84	1183.39	23.23	**9.6**
	Contig8050_at	AAN33181.1 e-113 major facilitator superfamily antiporter [Oryza sativa (japonica cultivar-group)]	57.08	14.00	314.70	7.08	**5.5**
	HV_CEa0013E09r2_at	AAG17016.2 2e-33 iron-phytosiderophore transporter protein yellow stripe 1 [Zea mays]	229.09	27.34	806.58	37.07	**3.5**
	Contig7377_s_at	AAK26773.1 2e-50 tonoplast membrane integral protein ZmTIP4-2 [Zea mays]	231.48	34.00	733.69	24.77	**3.2**
	HVSMEb0012O23r2_s_at	AAG17016.2 2e-26 iron-phytosiderophore transporter protein yellow stripe 1 [Zea mays]	486.27	66.62	1523.05	35.13	**3.1**
	Contig14345_at	AAF36688.1 6e-62 secretory carrier membrane protein [Oryza sativa]	272.95	34.66	849.91	62.70	**3.1**
	Contig5935_at	Q9XGY5 4e-012 Mitochondrial import inner membrane translocase subunit Tim13 gb|AAD39987.1|AF150080_1 small zinc finger-like protein [Oryza sativa]	314.41	75.05	943.99	53.95	**3.0**
	Contig12565_at	T48383 2e-34 uracil transporter-like protein - Arabidopsis thaliana	237.70	34.33	711.28	45.63	**3.0**
	Contig14945_at	gb|AAQ91200.1 4e-081 putative glutathione transporter [Zea mays]; 2. gb|AAO32313.1 4e-080 putative oligopeptide transporter protein [Oryza sativa (japonica cultivar-group	241.86	34.24	700.77	58.39	**2.9**
	Contig9662_at	NP_198006.1 2e-81 hexose transporter - like protein; protein id: At5g26340.1.	790.57	72.57	2045.38	158.61	**2.6**
	Contig26036_at	NP_200978.1 6e-31 (NM_125564) ABC transporter family protein; protein id: At5g61700.1 [Arabidopsis thaliana]	327.11	46.73	761.05	40.48	**2.3**
	Contig11005_s_at	dbj|BAC83311.1 0 putative sorbitol transporter [Oryza sativa (japonica cultivar-group)	1094.99	73.68	2522.56	137.07	**2.3**
	Contig12788_at	NP_174485.1 3e-78 secretory carrier membrane protein. putative; protein id: At1g32050.1	298.56	36.05	681.10	30.85	**2.3**
Transport facilitation	Contig9541_at	BAA93045.1 3e-73 (AB040667) nonclathrin coat protein zeta2-COP [Zea mays]	169.06	44.83	594.69	60.50	**3.5**
	Contig15386_at	NP_568944.1 5e-14 (NM_125583) copine - like protein; protein id: At5g61900.1	59.32	12.71	165.86	19.36	**2.8**
	Contig8507_at	AAG46163.1 2e-94 putative ADP-ribosylation factor [Oryza sativa]	531.20	92.94	1423.57	96.77	**2.7**
	Contig4458_at	AAM66112.1 1e-84 putative coated vesicle membrane protein [Arabidopsis thaliana]	735.72	84.84	1902.35	38.41	**2.6**
Vitamine	Contig455_s_at	BAC45141.1 e-104 putative thiamine biosynthetic enzyme [Oryza sativa (japonica cultivar-group)]	1976.94	206.28	7226.86	143.05	**3.7**
	HVSMEb0014C02r2_s_at	Q41739 .003 Thiazole biosynthetic enzyme 1-2. chloroplast precursor pir||S61420 thiamin biosynthesis protein thi1-2 - maize	69.79	22.35	247.99	14.06	**3.6**
Unspecified category	Contig11818_at	AAG13504.1 2e-85 putative cytochrome P450 [Oryza sativa (japonica cultivar-group)]/ gi|13878919|sp|Q42602|C892_ARATH Cytochrome P450 89A2	229.56	18.38	472.60	21.16	**2.1**

coding for proteins involved in protein export were three-fold over-expressed (Table 6). These up-regulations also suggest that protein translation and production is more important in the field than in the greenhouse. This could be due to a larger number of expressed genes coding for proteins with shorter half-life than the proteins present in greenhouse-grown plants. Therefore, the leaf cells would need a bigger capacity of protein synthesis and would increase the amount of chaperones and other proteins involved in protein synthesis.

Three genes involved in iron metabolism, one iron-phytosiderophore transporter gene and two other metal transporter genes were more expressed in the field than in the greenhouse (Table 6). Similarly, two genes involved in nitrate metabolism showed higher transcript levels. The higher expression of these eight genes could reflect the higher need of wheat leaves for nutrients and metals in the field compared to greenhouse conditions.

Nine genes involved in energy production, nine genes from the glucide metabolism and three sugar transporter genes showed similarly elevated expression in the field (Table 6). Genes from the thiamine biosynthesis pathway were also induced. Thiamine cofactor is often used by enzymes involved in the carbohydrate metabolism and high translation rates of these genes can then be correlated. Two genes involved in ubiquinone biosynthesis also showed enhanced expression in field-grown plant compared to the greenhouse-grown samples. This possibly reflects antioxidative response as tobacco plants over-producing ubiquinone are more resistant to oxidative and salt stresses (Ohara et al., 2004). Five genes involved in cell wall synthesis and five genes coding for integral membrane proteins were around three-fold up-regulated. This could be correlated with the stronger leaf phenotype in the field than in the greenhouse where leaves are softer and more fragile.

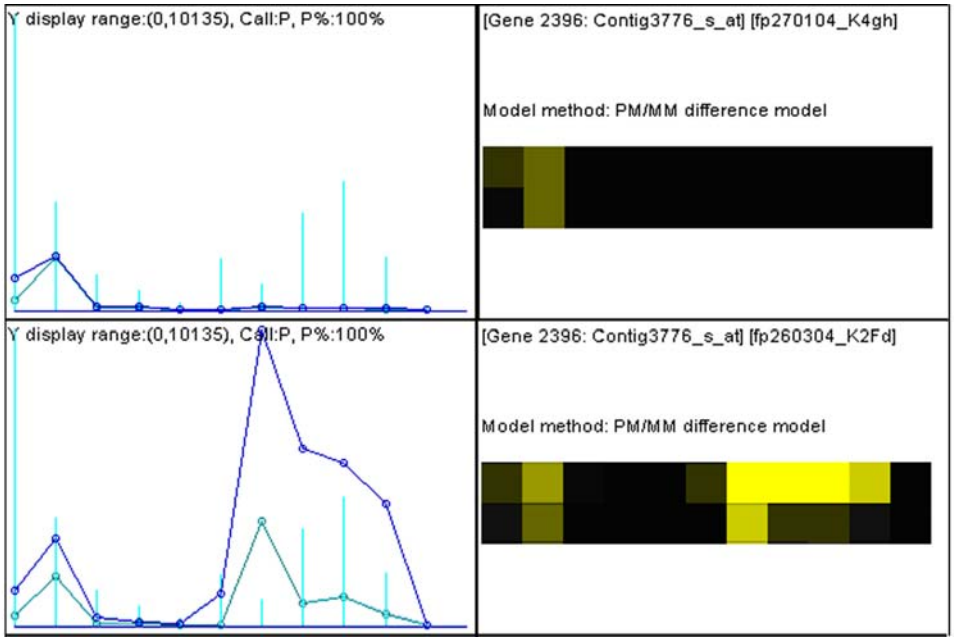


Figure 16: Hybridisation result, as in Figure 12, of the probe set contig3776_s_at, representing the putative lipid transfer protein gene. Bright signals were visible for five probes out the 11 for the field sample (K2Fd) whereas no hybridisation was visible for the same oligonucleotide probes for the greenhouse sample (K4gh). The first two probes as well as the seventh probe of this set showed non specific hybridisations for both conditions.

4.3.4 Phylogenetic analysis of the contig3776, a putative lipid transfer protein gene

The expression of contig3776, encoding a putative lipid transfer protein (LTP), was 92-fold higher in the field than in the greenhouse. As other LTP genes (*DIR1* in Arabidopsis and *IIG1* in maize) are possibly involved in defence signalling (Huang et al., 1998; Maldonado et al., 2002) the homology between these genes and the contig3776 from barley was studied in more details. Homologues of all three genes were found in all species studied. In total, 116 cDNAs similar to these three sequences were retrieved from the TIGR databases from Arabidopsis, maize, rice, barley and wheat (Table 7). We found that *IIG1* and contig3776 are more homologous to each other than to *DIR1*. Most of the LTP genes have an ORF that encodes proteins between 99 and 160 amino acids in a single exon structure. These short leucine- and proline-rich proteins contain highly conserved motifs such as the trypsine-α-amylase inhibitor domain. Eight cysteine residues are present in all LTP family homologues. Two myristoylation sites are present in most of the sequences as well as an amidation motif in the N-terminal part of the protein. A subset of 37 sequences representing the diversity within the LTP family was used to create a phylogenetic tree. After translation of the sequences, a phylogenetic tree was constructed to better asses the homology between the LTPs (Figure 17). This tree showed that the contig3776 protein is closer to IIG1 than to DIR1. Two groups were clearly distinct: one containing the sequences IIG1 and the contig3776, and the other one containing the sequences closely related to DIR1.

Twenty-three cDNA sequences were selected by blasting with either contig3776 or IIG1 against the cereal databases. The two sequences have an identity of 50%, although the lengths of the two proteins differ by 30 residues, the barley sequence being longer (159 residues); the identity level between the two sequences increases to 61% if the different sequence lengths are not considered. The wheat homologue AL820348, obtained from a 2-day post germination

Table 7: cDNA homologues to the contig3776, DIR1 and IIG1 sequences retrieved by blast search in TIGR databases of Arabidopsis, barley, maize, rice and wheat. Sequences in bold type represent the subset used for the phylogenetic tree creation. Sequences highlighted in blue correspond to common sequences found after two blast searches with contig3776 and IIG1 as query.

	DIR1	contig3776	IIG1
Arabidopsis	**AF342726-DIR1** AY062857 AY085224 **AY087218** BT002886 BT006510 BX832863 BX833987	**AAO23622** CAB78294 **AY093032** AF104328	**AY091005** AF412111
Barley	**BQ765331**	**BQ467852-TC121162-contig3776** AV912950-TC121164 BQ765319-TC121160 **CB869490-TC121163** BM816117-TC121159 **TC112284 -CA020259** TC121110 **TC109040** TC124193-BE413023 **TC119683 -BG343557** TC119806 TC126020 - BM817079	TC121161 -BQ467852 **BM816117-TC121159** **CB869490-TC121163** BQ765319-TC121160 AV912950-TC121164 **BQ467852-TC121162-contig3776** TC124193-BE413023 **TC112284 -CA020259**
Maize	**AY103938** BQ293701 **CD442815**	**TC234195 -BM080570** **TC220986 -AY108363** **TC222375 -AY108411** **TC234193 -AY105972** TC221071 -BM073347 TC220041 -BM332371 TC234194 -BQ619488 TC220043 -AY105236 TC219137 -CF061147 TC221070 -CF021142 TC220042 - CF021038 **TC219139 -AF001634** TC219136 -CF061669 TC220114 -AY106116	**AF001634-IIG1** AY104687-salt AY105236 CF021038 **AY105972** **AY108411** AB018587 **AB018588**
Rice	**AK105204**	**TC234598 -AK103618** **TC239377- AK062654** TC232030 -AK062911 CR292415 TC234297 - AC026758 **TC232028 -AK109149** TC232031 -AK062381 TC231004 - CAE05203.3 TC232034 -AY466109 TC232033 - AC026758 TC232032 -AC026758 TC243661 -AU101405 TC222481 -AC026758 **TC230993 -AL731610** BE039206	AC026758 **AK062654** **AK103618** AY466109 **AK058218** CR292415 AK062911 **AK062848** BE039206
Wheat	AL826197 **BF484978** **CA618888** **CA619036**	**TC182595-AL821523** **TC178419-AL820348** TC144646 -CA720574 TC144700 -CK166067 CK211882 **CK196743** BJ281358 BJ279286 BQ838475 **CK204391** CK200679 CK199480 CA662670 CA646396 CA601700 CA603419	**CA622455** **CA624634** CA616450 CA623157 CA613510 **CK196743** CK200565 CA601700 CA633656 **CK204391** CK199480

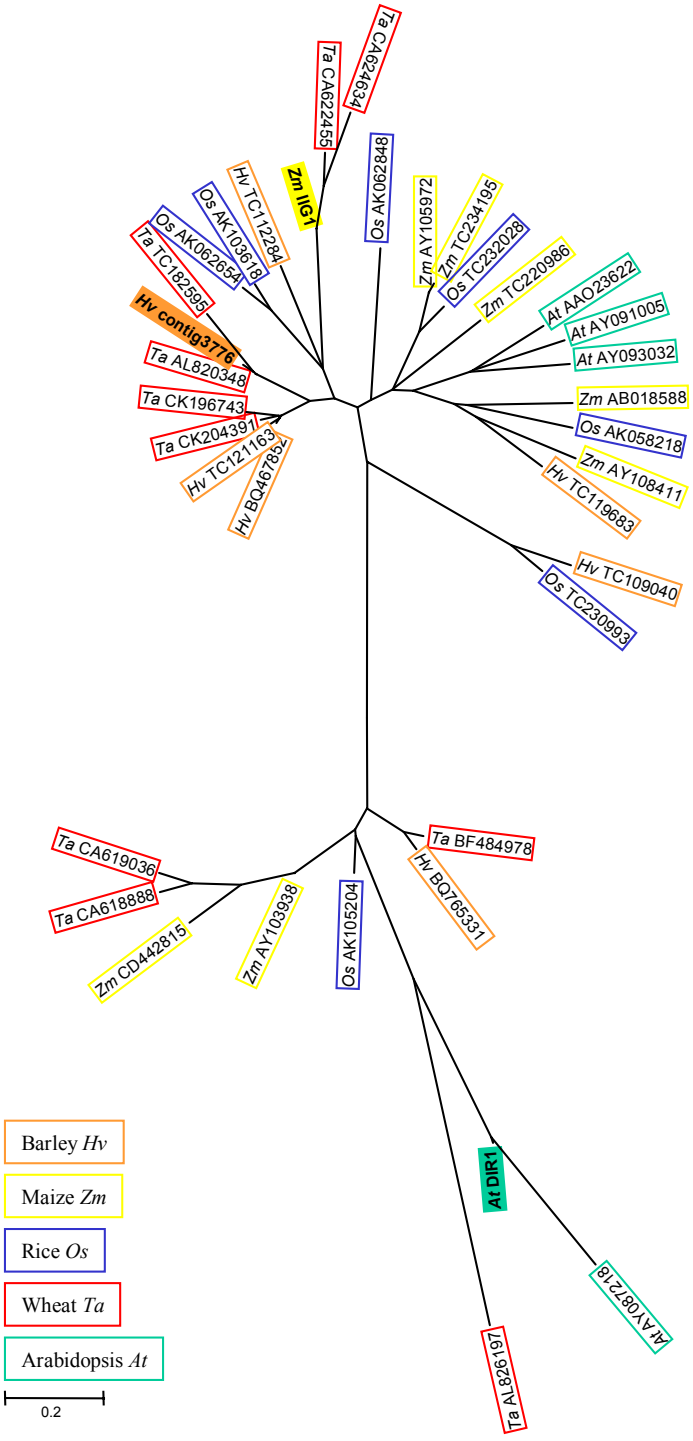


Figure 17: Phylogenetic tree of protein homologues to contig3776 protein, maize IIG1 and Arabidopsis DIR1 created with MEGA3 software using Minimum Evolution, Pairwise Deletion and Dayhoff's substitution matrix methods. Barley homologues are framed in orange, wheat in red, rice in blue, maize in yellow and Arabidopsis in green.

cDNA library, was the closest homologue to contig3776 with only 11 amino acid differences between the two sequences. The main difference was the presence of 5 additional amino acid residues in the barley sequence. Another wheat sequence (TC182595, coming from a cold-stressed seedling cDNA library) was also highly similar to contig3776 but this wheat protein has 12 residues more than the barley protein at the beginning of the sequence and the 12 last amino acids share few similarities. These three sequences are 76.3 % identitical. A sister-group composed of four sequences that were obtained by blasting either with contig3776 or with IIG1 against the wheat and barley databases were identical at 87.2%, possibly corresponding to paralogues in each species.

Two wheat sequences (CA634624 and CA622455, coming from 7-day-old leaf seedlings) were highly similar to IIG1 and form a subgroup in a bigger cluster containing two closely related rice sequences and a barley sequence. Sequences similar to IIG1 were often found to be the same as the ones obtained when using contig3776 as a query, reflecting their high homology (Table 7). However, corresponding proteins also grouped more often between species than within the same species, reflecting that these closely related proteins are conserved among cereal species.

The nine DIR1 homologues could be separated into two groups: one containing two wheat sequences (CA618888 and CA19036) and two maize sequences (AY1039381 and CD442815) that have high homologies with each other, mostly at the N-terminal part, and are between 16 and 47 residues longer than the other DIR1 homologues that form the second subgroup. In the tree, the wheat sequence AL826197 was the most similar cereal protein to DIR1 although it contains 50 residues more than DIR1. The rice homologue AK105204 was closer to DIR1 in length and identity. It belongs to the same cluster as the two Arabidopsis proteins (DIR1 and AY087218) and the wheat protein AL826197 (Figure 17). The low identity (6%) between the Arabidopsis and the cereal sequences in this subgroup could either reflect the divergence

between dicots and monocots, and/or that DIR1 has no true homologue in grasses. Nevertheless, the contig3776 could play a role similar to IIG1 as an early component in the response to stress (Huang et al., 1998).

4.3.5 GeneChip microarray analysis: Genes with stronger expression in the greenhouse

In the controlled greenhouse environment, 21 genes showed a higher expression than in the field (Figure 14B, Table 8). The two transcription factor genes *CCA1* and *LHY* (Figure 12), implicated in the circadian clock (Carre and Kim, 2002), were 9- and 16-fold more abundant than in field grown plants. However, this could be due to the time shift when the samples were harvested (late morning for the greenhouse samples, beginning of afternoon for the field samples). Interestingly, the RNAse S-like protein gene showed a similar 8-fold induction ratio as in the cDNA microarray experiment. The corresponding protein has no RNAse function and was shown to be light responsive unlike the dicots corresponding protein/gene (Gausing, 2000). Its over-expression could then be under the control of *LHY* or *CCA1*.

The uroporphyrin-III methyltransferase-like protein is involved in heme synthesis and its gene was over-expressed nearly seven fold (Table 8). The chlorophyll a/b-binding protein gene was also showing a six-fold higher expression compared to the field conditions but without statistical relevance because of higher intensity level in one of the three chips. However, this could mean that the light intensity was more important in the greenhouse.

Two genes involved in amino-acid metabolism, the asparaginase and the aspartate kinase genes, showed a three- and four-fold induction, respectively (Table 8). These genes are coding for enzymes involved in aspartate synthesis. Aspartate is a key amino-acid for lysine, threonine and methionine syntheses. The phosphoethanolamine methyltransferase gene was also higher expressed in the greenhouse than in the field. The corresponding enzyme methylates phosphoethanolamine using a methyl group from S-adenosylmethionine and has a key role in the biosynthesis of choline, a precursor of the osmoprotectant glycine betaine.

Table 8: Highly expressed genes in the greenhouse (GH) compared to conditions field (FD) determined by GeneChip analysis. Signal intensity means for the three replicates for each condition and corresponding standard deviation (se) and the fold change ratio between the two means are indicated.

Class	Probe Set	Putative function	GH mean	GH mean's se	FD mean	FD mean's se	**fold change**
Amino-acid	Contig8740_at	AAG28786.1 2e-71 (AF308474) asparaginase [Hordeum vulgare]	859.60	74.28	290.01	30.19	**-3.0**
Auxin	Contig2503_s_at	AAD32146.1 4e-65 Nt-iaa28 deduced protein [Nicotiana tabacum]	1490.77	221.40	438.84	42.54	**-3.4**
Cell wall	Contig10522_at	P04929 1e-05 histidine-rich glycoprotein precursor pir||KGZQHL [Plasmodium lophurae]	1075.32	84.37	263.35	41.34	**-4.1**
Chaperone	Contig17190_at	AAL83988.1 2e-36 (AF358772) putative heat shock protein [Oryza sativa]	1367.38	172.13	329.18	45.63	**-4.2**
Chlorophyll	Contig10779_at	BAB89483.1 6e-98 uroporphyrin-III C-methyltransferase-like protein [Oryza sativa (japonica cultivar-group)]	306.48	19.72	44.31	17.04	**-6.9**
	Contig628_x_at	gb|AAB18209.1 0 chlorophyll a/b-binding protein WCAB precursor [Triticum aestivum]	3859.21	848.30	723.06	132.12	**-5.3**
SAM	Contig2191_at	AAL40895.1 e-139 phosphoethanolamine methyltransferase [Triticum aestivum]	3879.27	268.75	1377.86	111.66	**-2.8**
Iron	Contig8185_at	JA0172 e-113 ferredoxin--nitrite reductase (EC 1.7.7.1) precursor - maize (fragment)	2439.13	306.20	559.47	70.38	**-4.4**
Kinase	Contig7534_at	BAC10350.1 e-106 (AP003818) putative serine/threonine kinase [Oryza sativa]	1067.07	42.10	423.73	35.20	**-2.5**
Lipid	HO08B11S_at	BAB91850.1 3e-05 (AP003350) putative fatty acid condensing enzyme CUT1 [Oryza sativa (japonica cultivar-group)]	170.12	8.80	42.42	11.96	**-4.0**
Metal	Contig3057_s_at	emb|CAE05547.1 0 OSJNBa0053B21.21 [Oryza sativa] 2. ref|NP_568974.1 2e-094 copper chaperone (CCH)-related [Arabidopsis thaliana]	353.72	22.61	93.99	12.87	**-3.8**
Signal transduction	Contig5185_at	AAF45043.1 e-118 RNase S-like protein precursor [Hordeum vulgare]	1812.22	116.48	220.50	51.05	**-8.2**
	Contig2433_s_at	CAB71336.1 e-112 putative acid signal transduction [Hordeum vulgare subsp. vulgare]	513.96	47.06	96.75	13.58	**-5.3**
	Contig13989_at	CAC85949.1 e-114 (AJ312330) dof zinc finger protein [Hordeum vulgare subsp. vulgare]	1855.12	251.74	545.33	44.40	**-3.4**
Sugar metabolism	Contig5572_at	AAG00180.1 e-121 (AF271995) phosphoenolpyruvate carboxylase [Oryza sativa]	801.86	68.57	208.59	44.23	**-3.8**
	Contig4635_at	NP_171617.1 e-110 pyruvate dehydrogenase E1 alpha subunit; protein id: At1g01090.1	520.23	89.66	138.31	32.44	**-3.8**
Transcription factor	Contig3873_at	CAD12767.2 5e-36 LHY protein [Phaseolus vulgaris]	4100.81	424.87	253.88	20.94	**-16.2**
	Contig3875_s_at	NP_566088.2 1e-23 (NM_130250) MYB-related transcription factor (CCA1); protein id: At2g46830.1, supported by cDNA: gi_1777442	2176.19	236.71	232.54	24.54	**-9.4**
	Contig8369_at	gb|AAD22495.3 5e-028 AF134116_1 APETALA2 protein homolog HAP2 [Hyacinthus orientalis]	613.35	150.93	135.82	24.34	**-4.5**
	Contig26393_at	NP_188825.1 2e-15 (NM_113083) PREG-like protein, putative; protein id: At3g21870.1 [Arabidopsis thaliana]	399.44	41.92	143.19	12.28	**-2.8**
Transport	Contig8740_at	NP_199567.1 4e-69 sodium-dicarboxylate cotransporter-like; protein id: At5g47560.1, supported by cDNA: 107593.	723.99	133.62	173.56	29.27	**-4.2**

4.4 Conclusion

An initial comparison of two different growth conditions on wheat gene expression using a microarray containing 600 cDNA probes had given new information about the reaction to environmental stress. Some genes were differentially expressed between the field and the greenhouse conditions. Most genes involved in defence response were over-expressed in the field and the RNAse S-like gene in the greenhouse. The use of the Barley1 GeneChip from Affymetrix demonstrated that there are many additional differentially expressed genes in the two conditions. E.g., the larger set of probes revealed that genes such as the lipid transfer protein gene were highly over-expressed in the field conditions. With the possibility of the GeneChip technique to detect changes for genes expressed at low levels, it was possible to identify several transcription factor genes that were not highly enough expressed for the detection in cDNA microarray. However, several genes that were present on both platforms have given similar results such as the RNAse S-like genes and most of the defence-related genes reflecting that the cDNA microarray technique is reliable for relatively highly expressed genes. Thus, the expression patterns obtained with the two techniques were correlated with detection sensitivity of the platforms.

As numerous transcription factor and ribosomal protein genes were induced by the field growth conditions, a more intense transcriptional activity seems to occur in this environment compared to the greenhouse conditions. The defence-related gene family as well as genes coding for proteins involved in protein synthesis were highly induced in the field compared to genes from primary metabolism that did not show major differential expression except for a few genes involved in sugar metabolism. We speculate that the transcription of all these genes contributes to a better adaptation of wheat to the field environment.

V. Common and distinct gene expression patterns induced by the herbicides 2,4-D, cinidon-ethyl, and tribenuron-methyl in wheat

Frédérique Pasquer, Jürg Zarn and Beat Keller

5.1 Abstract

In wheat, herbicides are mainly used to eliminate broadleaf weeds. Little is known about the changes induced in the metabolism of resistant plants after herbicide treatment. Here, we studied the impact of three herbicides 2,4-D, cinidon-ethyl, and tribenuron-methyl on the wheat transcriptome using cDNA microarrays. Gene expression of plants grown in a controlled environment or in the field was studied between 24 hrs and two weeks after treatments. Under controlled conditions, 2,4-D induced genes of the phenylpropanoid pathway early after treatment. This possibly reflects 2,4-D detoxification by its incorporation into the cell wall in lignol form. Cinidon-ethyl triggered peroxidase and defence-related gene expression under controlled conditions, probably because reactive oxygen species are released by photo-oxidation of protoporphyrin-IX. Furthermore, peroxidases could be involved in several steps of the cinidon-ethyl detoxification process. The same genes as in the controlled conditions were up-regulated in the field, albeit at a weaker level. These results show that cinidon-ethyl specifically induces genes involved in plant defence. Under controlled conditions, tribenuron-methyl did not change the expression profile early after treatment, but defence-related genes were up-regulated after one week. Sulfonylurea compounds such as tribenuron-methyl are specifically inhibiting the acetolactate synthase and are rapidly detoxified, but the activity of some resulting metabolites could explain later changes in gene expression. Finally, over-expression of the isopropylmalate synthase gene, involved in branched-chain amino acid synthesis, and of defence-related genes was observed in the field after the sulfonylurea treatment.

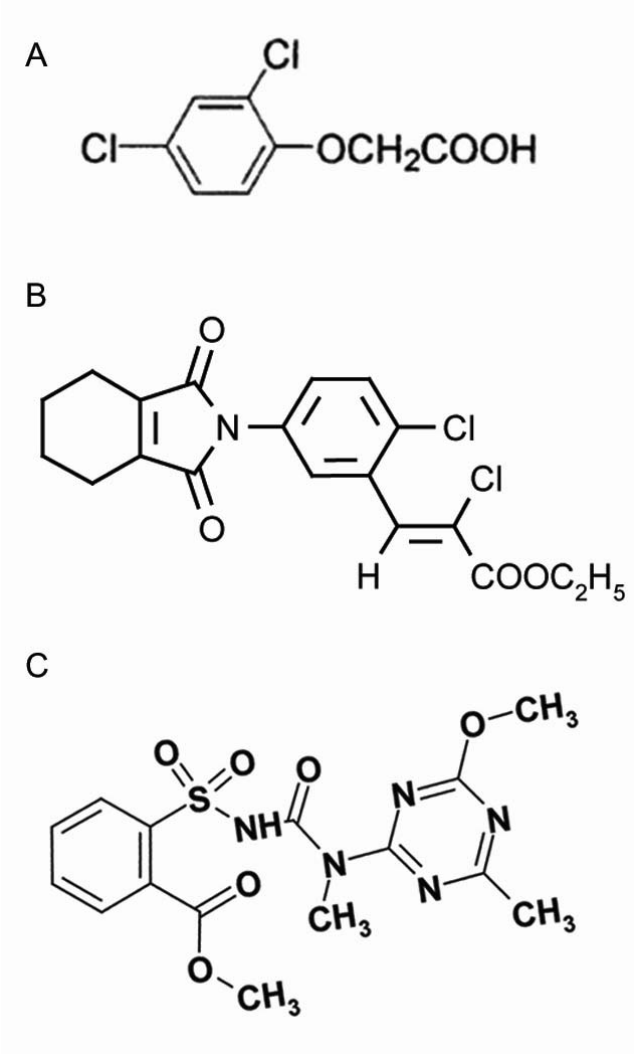


Figure 18: Chemical structure of the three herbicides used in our trials. A: 2,4-D, B: cinidon-ethyl, C: tribenuron-methyl.

5.2 Introduction

In the field, crops are competing with weeds for nutrients and light. To improve yield, selective herbicides against weeds are applied on crops that are themselves resistant to these compounds. Modern herbicides act very efficiently on enzymes which are specific to plants and absent in animal cells. These molecules are therefore not toxic for animals. More than 60% of the new herbicides are targeting chloroplast function or structures, and act, for instance, as inhibitors of electron transport, amino acid synthesis or synthesis of pigments (Wakabayashi and Boger, 1999). In resistant crops, these herbicides either fail to interact with the target enzyme, are poorly taken up and translocated and/or are detoxified (Forthoffer et al., 2001). Herbicide detoxification occurs by chemical transformation followed by compartmentalisation (Coleman et al., 1997). Structural modifications of the xenobiotics involve first hydrolysis or oxidation (phase I) followed by the conjugation to an amino acid, glutathione or glucidic group (phase II), resulting in a hydrophilic non-toxic metabolite. Such metabolites are then transported into the vacuole, incorporated as bound residues (phase III) or integrated into natural macromolecules (phase IV) (Skidmore, 2000).

Auxin-like herbicides are the oldest selective compounds for the control of broadleaf weeds. These synthetic growth regulators mimic the plant hormone indol-3-acetic acid (IAA) and induce uncontrolled cell elongation, chloroplast swelling, followed by general disruption of membranes (Devine et al., 1993b; Sterling and Hall, 1997). Cereals are resistant to auxin-like herbicides such as 2,4-D (2,4-dichlorophenoxyacetic acid, Figure 18A). The distinct phloem anatomy in dicots and monocots can explain part of the selective action. In monocots, the phloem is surrounded by sclerenchyma tissue only and neither cambium nor pericycle are present. In contrast, these two last tissues are present in dicots and are auxin-sensitive. In addition to the absence of these tissues in monocots, the presence of intercalary meristem between stem and leaves could prevent toxicity in cereals because of a reduced translocation

rate (Sterling and Hall, 1997). However, selectivity does not only result from differences in uptake or translocation as similar levels of herbicide accumulation can be found in resistant and susceptible species (Dexter et al., 1971). Distinct metabolisation and detoxification pathways of the herbicide might explain its selective action. For instance, in wheat, fast and irreversible metabolisation of the auxin-type herbicides through a ring hydroxylation resulting in 4-OH-2,5-D ("NIH" shift), probably catalysed by a cytochrome P450 oxidase, can explain the resistance of the plant (Devine et al., 1993b; Coupland, 1994). This first detoxification step is followed by a conjugation to a glucoside or an amino acid in wheat (Broadhurst et al., 1966; Bristol et al., 1977). The resulting conjugate does not show any herbicidal activity anymore and is either compartmentalised or incorporated into bound macromolecules (Scheel and Sandermann, 1981).

The bleaching herbicide cinidon-ethyl (ethyl-2-chloro-3-[2-chloro-5-(1,3-dioxo-4,5,6,7-tetrahydroisoindol-2-yl)phenyl] acrylate, Figure 18B) is an isoindoldione that inhibits the protoporphyrinogen oxidase (protox, EC 1.3.3.4), a key-enzyme for synthesis of tetrapyrolic molecules such as hemes, phytochromes and chlorophylls (Grossmann and Schiffer, 1999; Wakabayashi and Boger, 1999). This chloroplastic enzyme, protox, oxidises protoporphyrinogen (protogen) to form protoporphyrin-IX (proto-IX). Inhibition of protox activity results in accumulation of the reaction product, proto-IX, due to an extra-chloroplastic oxidation (Lee et al., 1993). Proto-IX is then photo-oxidised, producing reactive oxygen species (ROS) which are responsible for the lipid peroxidation, membrane destruction and pigment degradation leading to bleaching of the leaves (Asami and Yoshida, 1999; Wakabayashi and Boger, 1999). Although wheat protox and the protox from sensitive species have the same sensitivity to the compound *in vitro*, cereals are tolerant to this type of herbicide (Grossmann and Schiffer, 1999). Resistance could be due to destruction of protogen to non-porphyrin compounds (Jacobs et al., 1996), to metabolisation of proto-IX and/or to

counteraction of the peroxidative effects by antioxidative enzymes (ascorbate peroxidase, catalase, glutathione reductase) coupled with the degradation of the herbicide (Grossmann and Schiffer, 1999; Knorzer and Boger, 1999).

Another group of herbicides commonly used because of low toxicity for animals are the sulfonylurea. These compounds, such as tribenuron-methyl (methyl-2-[N-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-N-methyl-carbamoylsulfamoyl]benzoate, Figure 18C), inhibit the acetolactate synthase (ALS), the key enzyme of branched-chain amino acid synthesis. Exogenous complementation with branched-chain amino acids can completely reverse the herbicidal activity of sulfonylurea (Devine et al., 1993c). Therefore, these herbicides are very selective and are active at very low concentration (2g/ha). Resistance of cereals is partially due to low uptake and translocation of the xenobiotic (Saari et al., 1994) but this does not fully explain the 1000-fold increased resistance compared to sensitive species (Brown, 1990). The barley and wheat ALS enzymes are sensitive to sulfonylurea herbicides *in vitro* (Sweetser et al., 1982; Hall et al., 1994). However, *in planta*, rapid N-demethylation, arylhydroxylation of the phenyl ring followed by glycosylation and cleavage of the sulfonylurea bond form non-toxic metabolites and provide the basis for this non-target site based resistance (Anderson et al., 1989; Roberts, 1998b; Owen, 2000). Metabolisation of nearly 100% of chlorsulfuron, another sulfonylurea, happens in 24 hrs in wheat whereas this herbicide remains intact in sensitive plants (Sweetser et al., 1982).

Most of the information about the effects of herbicides on plant metabolism comes from chemical or biochemical studies on the xenobiotic degradation and little is known on the expression of genes involved in the detoxification processes (Roberts, 1998a; Grossmann, 2000a). Most of the enzymes and pathways induced upon xenobiotic application remain unknown (Aizawa and Brown, 1999). In order to determine the effect of herbicides on wheat metabolism, we have studied gene expression profiles upon treatment with cinidon-ethyl, 2,4-

D and tribenuron-methyl. Gene expression patterns of wheat leaves treated with the herbicides were compared with untreated plants grown under the same conditions. Specific gene expression changes were observed after treatment in the controlled environment and could be related to distinct detoxification systems for each compound. Results from the field trial showed different expression patterns compared to the experiment under controlled conditions. This indicates that the wheat plants differentially react to the xenobiotics, depending on the environmental conditions.

5.3 Materials and methods

5.3.1 Plant material and herbicide treatments

Seeds of spring wheat (*Triticum aestivum* L., variety Greina) were grown in a phytotron (16 hrs light/ 20°C, 8 hrs night/ 16°C, 4 seeds per pot). At growth stage 29 (Tottman, 1987), plants were treated with either 2,4-D (Gesin®, Syngenta (Basel, Switzerland) at the concentration of 2.5l/ha), cinidon-ethyl (Lotus®, BASF (Wädenswil, Switzerland) at the concentration of 0.25l/ha) or tribenuron-methyl (Express®, Dupont, Geneva, Switzerland) at the concentration of 25g/ha), as recommended by manufacturers. Other plants were kept as untreated controls. In the field, the plants were sown in 5-row plots (1.3 m wide, 1.2 m long, approximately 50 seeds/row) near Zürich, Switzerland, at the Swiss Federal Research Station for Agroecology and Agriculture (FAL Reckenholz, 440m above sea level). For each treatment, 4 plots were sprayed with one herbicide, following the same protocol as for the treatment in the controlled conditions. Four additional plots were left untreated and used as control. For both trials, leaves were harvested at 24 hrs, 72 hrs, one and two weeks after treatment.

5.3.2 cDNA microarray analysis

A microarray with 600 barley cDNAs was prepared as previously described in chapter 2. RNA samples from control plants were labelled with Cy3-dCTP and treated RNA samples with Cy5-dCTP (Amersham Biosciences, Otelfingen, Switzerland) during reverse transcription (Reymond et al., 2000).

After overnight hybridisation at 65°C and subsequent washings, microarray slides were scanned using a ScanArray 5000 (PerkinElmer Life Sciences, Rodgau-Jügesheim, Germany) at the resolution of 10μm/pixel and with settings adjusted to obtain similar signal intensity levels for both channels. Pictures were analysed by Imagene 4.1 software (BioDiscovery Inc., Los Angeles, USA). Normalisation of the signal intensities between the two channels was performed using the global method and between the triplicate slides by scale normalisation (Yang et al., 2002). One-class analysis from the Excel add-in Significance of microarray analysis (SAM) (Tusher et al., 2001) was performed for each experimental condition in order to detect the differentially expressed genes and the false discovery rate (FDR) counterpart. Delta value was set to have only one gene falsely detected among the differentially expressed genes (Samimi et al., 2004). As the numbers of differentially expressed genes were rather low, the FDR reached relatively high levels (up to 43%, see Appendix 8.5). For three conditions (72 hrs after cinidon-ethyl treatment under controlled conditions, 24 hrs after 2,4-D treatment in the field and one week after tribenuron-methyl treatment in the field), no gene was detected as differentially expressed according to our filtering criteria. Cluster analyses were carried out using the Genesis software (Sturn et al., 2002).

5.3.3 Northern blot analysis

The results from microarray experiments were validated by RNA blot analysis when indicated in the text. Total RNA (40 μg) was separated by electrophoresis and transferred to a nylon

Table 9: Differentially expressed genes in plants grown under controlled conditions 24 hrs after treatment with either 2,4-D, cinidon-ethyl (C), and tribenuron-methyl (T). Intensity ratios of genes determined to be differentially expressed by SAM analysis are in bold type. The positive values indicate gene induction and negative values indicate gene repression.

Gene ID	Accession number	Putative function	2,4-D	C	T
HVSMEg0001A02f	BE230858	homologue to UP|Q84N28 (Q84N28) Caffeic acid O-methyltransferase	**4.4**	3.7	**3.0**
HVSMEg0015D01f	BE455799	UP|Q9MAY8 (Q9MAY8) Endo-1 4-beta-glucanase Cel1	**3.7**	1.0	1.9
HVSMEg0002G13f	AW982232	UP|Q9MAY8 (Q9MAY8) Endo-1 4-beta-glucanase Cel1	**2.7**	-1.3	1.7
HV_CEb0018M20f	BE519721	similar to UP|O23254 (O23254) Serine hydroxymethyltransferase	**2.6**	1.5	1.7
HV_CEb0006A03f	BE215152	UP|O04876 (O04876) Phenylalanine ammonia-lyase (Fragment) (EC 4.3.1.5)	**2.6**	1.9	1.5
HV_CEb0004M23f	BG299484	UP|Q7XTK5 (Q7XTK5) IAA1 protein	**2.5**	-1.0	1.4
HV_CEb0022L14f[a]	BE216724	homologue to UP|Q8W4U9 (Q8W4U9) Clathrin assembly AP17-like protein	**2.4**	**2.0**	1.3
HV_CEb0003P13f	BE214612	homologue to UP|Q6K1Q8 (Q6K1Q8) Putative phenylalanine ammonia-lyase	**2.4**	1.8	1.6
HV_CEb0017H19f	BE558391	similar to UP|Q94IP1 (Q94IP1) Cinnamic acid 4-hydroxylase (EC 1.14.13.11)	**2.4**	2.9	1.6
HVSMEg0008F21f	BG344249	similar to UP|Q9LGS7 (Q9LGS7) Putative cytochrome P450	**2.3**	-1.0	1.6
HVSMEg0001P11f	BF261118	similar to UP|Q9FYP0 (Q9FYP0) Putative peroxidase	**2.0**	1.1	1.5
HVSMEg0012F21f	BE060460	homologue to UP|Q84P58 (Q84P58) Adenosine kinase-like protein (Fragment)	**2.0**	1.5	1.5
HVSMEh0081M04f	BE193575	homologue to GB|CAA75793 sucrose synthase 2 {Hordeum vulgare}	**1.9**	1.1	1.3
SFR009.H10F990514	BE438015	homologue to UP|Q84P58 (Q84P58) Adenosine kinase-like protein (Fragment)	**1.8**	1.6	1.3
HV_CEb0003P20f	BE214619	UP|CHS1_HORVU (P26018) Chalcone synthase 1	**1.8**	-1.4	1.8
SFR009.E05F990511	BE437975	similar to UP|O23254 (O23254) Serine hydroxymethyltransferase	**1.8**	1.3	1.3
HVSMEg0003O15f	AW982677	UP|CHS1_HORVU (P26018) Chalcone synthase 1	**1.8**	-1.1	1.6
HVSMEg0017G18f	BE231181	homologue to UP|Q75HE6 (Q75HE6) Putative methylenetetrahydrofolate reductase	**1.7**	1.7	1.2
HVSMEh0090J03f	BE195825	UP|METK_HORVU (P50299) S-adenosylmethionine synthetase 1	**1.7**	2.0	1.6
SFR004.G11F990621	BE437539	homologue to UP|RL11_MEDSA (P46287) 60S ribosomal protein L11 (L5)	**1.7**	1.2	1.1
HVSMEg0008B21f	BG344205	UP|METK_HORVU (P50299) S-adenosylmethionine synthetase 1	**1.6**	1.6	1.5
SFR008.F10F990628	BE437899	UP|G3PC_HORVU (P08477) Glyceraldehyde-3-phosphate dehydrogenase cytosolic (Fragment)	**1.6**	1.6	1.1
HVSMEg0002O09f	AW982323	homologue to UP|Q9LKM0 (Q9LKM0) Nucleoside diphosphate kinase (EC 2.7.4.6)	**1.6**	-1.1	1.2
HVSMEg0002O22f[a]	BE231062	homologue to UP|Q9LKM0 (Q9LKM0) Nucleoside diphosphate kinase (EC 2.7.4.6)	**1.6**	1.0	1.2
HVSMEh0100N24f	BE601995	homologue to UP|Q43638 (Q43638) Heat-shock protein precursor	**1.6**	1.0	1.0
HVSMEh0090M09f	AB029456	UP|Q9LRJ0 (Q9LRJ0) Glucose-6-phosphate dehydrogenase	**1.6**	2.1	1.2
HVSMEg0008I03f	AW983171	homologue to UP|Q8LMR0 (Q8LMR0) Putative phosphoserine aminotransferase	**1.6**	1.7	1.1
HVSMEg0003I12f	AW982536	homologue to UP|Q6RK07 (Q6RK07) UDP-glucose dehydrogenase	**1.5**	1.4	1.4
HV_CEb0001J24f	BE213910	similar to UP|Q9AQZ5 (Q9AQZ5) Putative heat-shock protein	**1.5**	1.4	1.3
HVSMEh0094B02f	BE454512	UP|METK_HORVU (P50299) S-adenosylmethionine synthetase 1	**1.5**	1.8	1.4
SFR009.E09F990628	BE437979	similar to UP|Q6YV24 (Q6YV24) Putative carbamoyl-phosphate synthetase small subunit	**1.5**	1.2	1.1
HVSMEg0019C14f	BE455709	similar to UP|Q9S834 (Q9S834) ATP-dependent Clp protease subunit ClpP (NClpP1)	**1.5**	-1.1	1.1
HV_CEb0021P01f	BE519980	UP|Q40068 (Q40068) Peroxidase (EC 1.11.1.7)	1.2	**14.1**	-1.4
HVSMEg0005I10f	BG343356	UP|PDI_HORVU (P80284) Protein disulfide-isomerase precursor (PDI)	1.2	**2.0**	1.1
HV_CEb0002C16f	BE214080	UP|ENPL_HORVU (P36183) Endoplasmin homolog precursor	1.1	**3.0**	1.3
HV_CEb0009I06f	BE216122	similar to UP|Q9XEN6 (Q9XEN6) Chitinase IV	1.1	**3.5**	1.0
wir1c	TARNAWIR1	UP|Q41581 (Q41581) WIR1 protein	1.0	**6.5**	-1.0
HVSMEh0088K24f	BE195244	UP|Q42839 (Q42839) Chitinase (EC 3.2.1.14 EC 3.2.1.14)	-1.0	**2.2**	-1.1
HV_CEb0003B05f[a]	BE214500	homologue to UP|WIRA_WHEAT (Q01482) WIR1A protein	-1.0	**5.3**	-1.1
HV_CEb0006E10f	BE215247	weakly similar to UP|Q9LWJ3 (Q9LWJ3) Similar to ethylene-forming-enzyme-like dioxygenase	-1.1	**6.6**	-1.2
HV_CEb0010G19f	BE216411	UP|E13B_HORVU (P15737) Glucan endo-1 3-beta-glucosidase GII precursor	-1.1	**2.8**	-1.2
HV_CEb0024H02f	BE559387	UP|Q43764 (Q43764) Chitinase (EC 3.2.1.14)	-1.1	**6.8**	-1.2
HV_CEb0021P03f[a]	BE519980	UP|Q40068 (Q40068) Peroxidase (EC 1.11.1.7)	-1.1	**4.0**	-1.2
pox381	X56011	UP|Q43212 (Q43212) Peroxidase precursor (EC 1.11.1.7)	-1.2	**17.8**	-1.5
HV_CEb0003A01f	BE214283	UP|Q43764 (Q43764) Chitinase (EC 3.2.1.14)	-1.2	**8.5**	-1.2
HV_CEb0003K12f	BE214507	UP|P93180 (P93180) Pathogenesis-related protein 4 precursor	-1.2	**4.4**	-1.2
SFR008.G11F990510	BE437912	homologue to UP|P93402 (P93402) Aspartate kinase-homoserine dehydrogenase precursor (EC 2.7.2.4 EC 1.1.1.3)	-1.2	-1.6	**-1.7**
wir232	TATHAU	UP|Q94F70 (Q94F70) Thaumatin-like protein	-1.2	**9.0**	-1.7
HVSMEh0101A21f	BE602672	UP|CHI1_HORVU (P11955) 26 kDa endochitinase 1 precursor (EC 3.2.1.14)	-1.2	**4.2**	-1.4
HV_CEb0006J08f	BE215358	UP|PR1A_HORVU (P32937) Pathogenesis-related protein 1A/1B precursor	-1.3	**6.0**	-1.6
gluc2	TC225609	UP|Q9XEN5 (Q9XEN5) Beta-1 3-glucanase	-1.3	**3.5**	-1.6
HV_CEb0010L20f	BE216529	UP|PR1_HORVU (Q05968) Pathogenesis-related protein 1 precursor	-1.5	**5.6**	**-1.8**

[a] : clones that have not given the same blastn result as in the database

membrane as previously described (Feuillet et al., 1997). The labelled probes (actin, peroxidase (pox381), chitinase (HV_CEb0003A01f), *WIR1c*, *PR 1a/1b* (HV_CEb0006J08f), *WCI2* and IAA1 (HV_CEb0004M23f)) were prepared using standard procedures (Sambrook et al., 1989) with clones previously used as templates for the barley cDNA microarray. The RNA blots were analysed using Biomax MS-1 film (Kodak, Lausanne, Switzerland).

5.4 Results

5.4.1 Alteration of gene expression after treatment with 2,4-D

Thirty-one out of the 600 genes present on the chip were up-regulated 24 hrs after treatment with 2,4-D in plants grown under controlled conditions (false discovery rate (FDR) of 3%) as shown in Table 9 and Figure 19A. The 2.5-fold up-regulation of a gene encoding an auxin-induced protein was confirmed by Northern blot analysis (Figure 20G). Genes involved in the phenylpropanoid pathway (lignin and flavonoid biosyntheses): phenylalanine ammonia-lyase (PAL) gene, the caffeic acid O-methyltransferase (COMT) gene, the cinnamate 4-hydroxylase (C4H) gene, the chalcone synthase gene and S-adenosylmethionine synthetase gene, as well as a peroxidase gene, were also significantly induced. The caffeoyl CoA O-methyltransferase (CCOMT) and ferulate-5-hydroxylase (F5H) genes from the phenylpropanoid pathway did not show differential expression. Two β-1,4-endoglucanase (E.C. 3.2.1.4) genes were also up-regulated. The cytochrome P450 gene, similar to a fatty acid hydroxylase cytochrome P450 86A1 gene, showed a two-fold increase of expression. After 72 hrs, a peroxidase gene and an iron/ascorbate oxidoreductase gene were showing slight up-regulation (Appendix 8.6). One week after treatment, the gene coding for the RUBISCO small subunit and a cysteine proteinase gene were nearly two-fold down-regulated (Table 10). The flavanone 3-hydroxylase gene was down-regulated two weeks after treatment (Appendix 8.7).

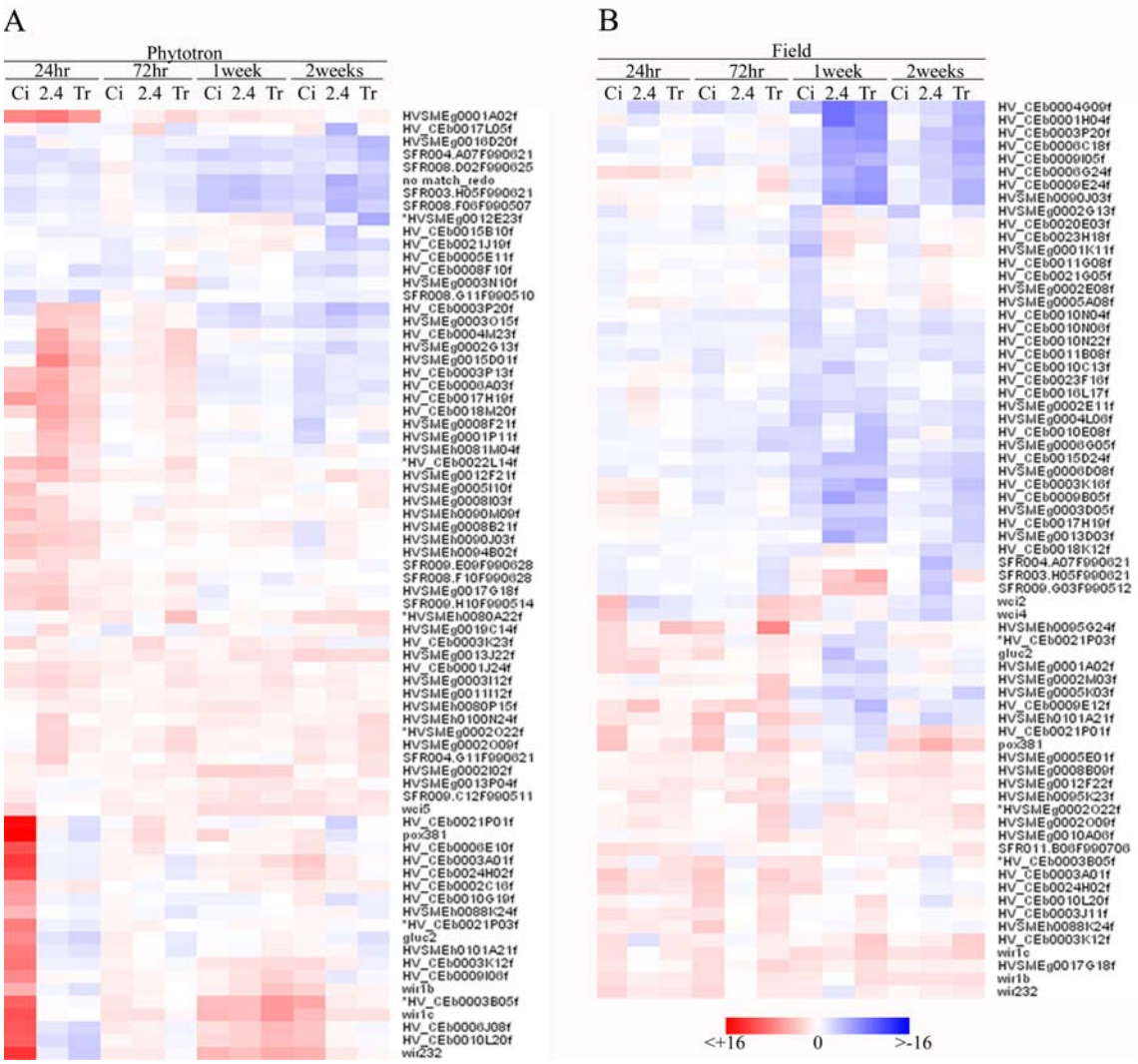


Figure 19: Hierarchical clustering of genes showing differential expression after treatment with cinidon-ethyl (Ci), 2,4-D (2.4) and tribenuron-methyl (Tr), displayed by the software Genesis. A: Treatments in the controlled conditions, B: Treatments in the field. The colour scale bar represents the ratio values. Genes with higher expression level after treatment compared to untreated plants appear in red; those with lower expression level appear in blue. These clusters were made with genes that showed differential expression for at least one time point (Tables 9 to 13).

In the field trial, no significant change of gene expression was observed after 24 hrs (Table 11, Figures 19B and 21), and only a thionin gene was up-regulated after 72 hrs (Table 12). After one week, a kinase gene was down-regulated as well as two sugar transporter genes, the PEPC gene and the fructose bisphosphate aldolase gene. Furthermore, the S-adenosylmethionine synthetase, C4H and chalcone synthase genes involved in flavonoids synthesis, a β-1,3-endoglucanase gene, a heat shock factor gene and a HSP 90 gene were also all repressed between 1.6 and 3-fold after one week (Table 13). The RUBISCO gene and chlorophyll a/b binding protein gene were down-regulated after two weeks (Appendix 8.8).

5.4.2 Alteration of gene expression after treatment with cinidon-ethyl

Several studies have demonstrated that inhibition of the protox enzyme by bleaching herbicides surprisingly resulted in the accumulation of the reaction product and not of its substrate due to an extra-chloroplastic conversion of protoporphyrinogen-IX to protoporphyrin-IX by a protox-like enzyme (Jacobs and Jacobs, 1993; Retzlaff and Boger, 1996). The wheat protox enzyme showed the same herbicide sensitivity as enzymes from susceptible weed species *in vitro* and rapid metabolisation was shown to confer resistance to the herbicide (Grossmann et al., 1999). In our study in wheat, protox gene expression was not changed after spraying with cinidon-ethyl confirming that the wheat resistance does not come from the over-expression of the gene encoding the target enzyme.

A total of 20 genes showed an altered expression 24 hrs after cinidon-ethyl treatment in controlled conditions. The majority of them (18 out of 20) belonged to the defence-related gene family (Table 9, Figure 19A). Three peroxidase genes were over-expressed up to nearly 20-fold. Five chitinase, two β-1,3- endoglucanase (E.C. 3.2.1.39), the PR 1, 1a/1b, 4, the WIR 1a, 1c and 232, and endoplasmin genes also showed statistically significant induction (between 2.2 and 9 fold-up, FDR of 4%). Furthermore, the ethylene forming-enzyme-like

Table 10: Differentially expressed genes in plants grown under controlled conditions one week after treatment with either 2,4-D, cinidon-ethyl (C), and tribenuron-methyl (T). Intensity ratios of genes determined to be differentially expressed by SAM analysis are in bold type. The positive values indicate gene induction and negative values indicate gene repression.

Gene ID	Accession number	Putative function	2,4-D	C	T
wir1c	TC149589	homologue to UP|Q41581 (Q41581) WIR1 protein	2.0	**2.1**	**3.0**
HV_CEb0003B05f[a]	BE214500	homologue to UP|WIRA_WHEAT (Q01482) WIR1A protein	2.0	**2.0**	**3.0**
wir232	TC136076	UP|PR1C_HORVU (P32938) Pathogenesis-related protein 1C precursor	2.0	**2.1**	**2.5**
HV_CEb0006J08f	BE215358	UP|PR1A_HORVU (P32937) Pathogenesis-related protein 1A/1B precursor	1.7	1.4	**2.1**
HV_CEb0010L20f	BE216529	UP|PR1_HORVU (Q05968) Pathogenesis-related protein 1 precursor	1.7	1.5	**2.0**
HVSMEg0002I02f	AW982245	UP|CAT2_HORVU (P55308) Catalase isozyme 2 (EC 1.11.1.6)	1.6	**1.6**	1.6
wci5	TC139571	similar to PIR|T06278 benzothiadiazole-induced protein (clone WCI-5) - wheat {Triticum aestivum}	1.4	**1.4**	1.4
HV_CEb0003K12f	BE214507	UP|P93180 (P93180) Pathogenesis-related protein 4 precursor	1.4	1.3	**1.5**
wir1b	TC130810	homologue to UP|WIRA_WHEAT (Q01482) WIR1A protein	1.4	1.2	**1.9**
SFR009.C12F990511	unknown	unknown	1.4	**1.2**	1.2
HVSMEh0080P15f	BU926883	similar to UP|Q9SWE2 (Q9SWE2) Alanine:glyoxylate aminotransferase 2 homolog	1.3	**1.3**	1.2
HVSMEg0013P04f	BE060944	UP|Q8GTR5 (Q8GTR5) BZIP transcription factor ZIP1	1.3	**1.2**	1.3
HVSMEg0011I12f	BE060120	similar to UP|Q93Y60 (Q93Y60) Putative chorismate mutase	1.2	1.2	1.2
SFR004.A07F990621	BE437465	similar to UP|Q75I52 (Q75I52) Expressed protein	**-1.5**	-1.6	-1.5
SFR008.F06F990507	BE437895	PIR|T05920 probable cysteine proteinase - barley (fragment) {Hordeum vulgare} (EC 3.4.22.-)	**-1.7**	-1.8	-1.5
EST1-15	BE438154	homologue to GB|BAC10351 unknown protein {Oryza sativa (japonica cultivar-group)}	**-1.9**	-1.7	-1.6
SFR003.H05F990621	BE437451	GP|11990901 ribulose-1 5-bisphosphate carboxylase/oxygenase small subunit {Triticum aestivum}	**-2.0**	-1.8	-1.6

[a] : clones that have not given the same blast result as in the database.

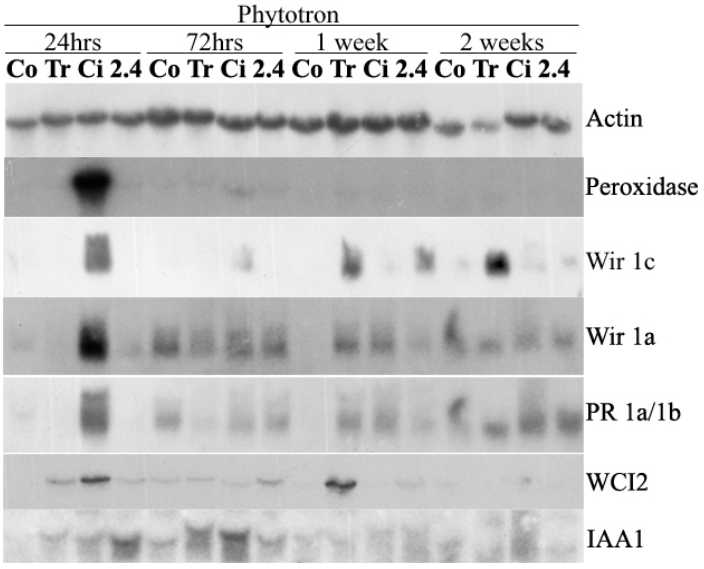


Figure 20: RNA blot analysis showing differential expression 24hrs, 72 hrs, one and two weeks after herbicide treatment of wheat in the controlled conditions trial. Co: control, Tr: tribenuron-methyl, Ci: cinidon-ethyl, 2,4: 2,4-D. Seven labelled probes were used: actin gene, as quality control of the blots, peroxidase gene (pox381), WIR 1c gene, WIR 1a gene, pathogenesis related PR 1a/1b gene (HV_CEb0006J08f), WCI2 gene, IAA1 gene (HV_CEb0004M23f).

dioxygenase gene was also significantly over-expressed. Genes from the phenylpropanoid pathway: PAL, COMT, C4H, 4-coumarate-CoA ligase (4CL), and chemically induced genes WCI 2, 4 and 5 were also between 1.6 and four fold up-regulated compared to untreated plants but this was significant only with a higher FDR (9%). All these alterations were transient as none of these genes showed differential regulation at later time point, i.e. 72 hrs after treatment. However, defence-related genes (*WIR 1a*, *1c*, *WCI5*, catalase and thaumatin-like genes) were again two-fold over-expressed after one week (Table 10). These results were confirmed by Northern blot analysis for one peroxidase gene, and the PR 1a/1b, WCI 2, WIR 1a and 1c genes (Figure 3).

In the field, the WCI 2 and 4 genes, three peroxidase genes, three chitinase genes and other PR genes were significantly up-regulated 24 hrs after treatment (Table 11, Figures 19B and 21). Due to constitutively high expression of defence-related genes, the induction factor was lower compared to the controlled conditions. Peroxidase and chitinase genes were similarly over-expressed after 72 hrs (Table 12, Figure 19B). After one week, seven kinase genes, two acid phosphatase genes as well as two sugar transporter genes were down-regulated (Table 13). A 1,4-β-glucanase gene was down-regulated after two weeks (Appendix 8.8).

5.4.3 Alteration of gene expression after treatment with tribenuron-methyl

The treatment with tribenuron-methyl resulted in few changes in gene expression in the plants grown under controlled conditions (Figure 19A). Only the caffeic acid O-methyltransferase gene showed a three-fold up-regulation after 24 hrs (Table 9). A PR B1 gene was slightly down-regulated at the same time. The auxin induced protein (IAA1) gene, two kinase genes and a histone H4 gene were up-regulated after 72 hrs (Appendix 8.6). Interestingly, seven defence-related genes (WIR 1a, 1b, 1c, PR1, 1a/1b, 4 and thaumatin-like) were significantly over-expressed one week after treatment (Table 10, Figure 20). The chalcone

Table 11: Differentially expressed genes in the field trial 24 hrs after treatment with either 2,4-D, cinidon-ethyl (C), and tribenuron-methyl (T). Intensity ratios of genes determined to be differentially expressed by SAM analysis are in bold type. The positive values indicate gene induction and negative values indicate gene repression.

Gene ID	Accession number	Putative function	2,4-D	C	T
wci2	TAU32428	UP|Q41520 (Q41520) Lipoxygenase (Fragment) (EC 1.13.11.12)	0.6	**2.1**	0.8
pox381	X56011	UP|Q43212 (Q43212) Peroxidase precursor (EC 1.11.1.7)	1.1	**1.9**	1.3
wci4	TAU32430	homologue to UP|Q41522 (Q41522) Thiol protease	0.8	**1.8**	0.8
HV_CEb0021P01f	BE519980	UP|Q40068 (Q40068) Peroxidase (EC 1.11.1.7)	1.1	**1.8**	1.1
HV_CEb0003A01f	BE214283	UP|Q43764 (Q43764) Chitinase (EC 3.2.1.14)	1.3	**1.7**	1.4
HV_CEb0024H02f	BE559387	UP|Q43764 (Q43764) Chitinase (EC 3.2.1.14)	1.3	**1.6**	1.3
HVSMEg0001A02f	BE230858	homologue to UP|Q84N28 (Q84N28) Caffeic acid O-methyltransferase	1.6	**1.5**	1.1
HV_CEb0021P03f[a]	BE519980	UP|Q40068 (Q40068) Peroxidase (EC 1.11.1.7)	1.1	**1.5**	1.1
wir232	TATHAU	UP|Q94F70 (Q94F70) Thaumatin-like protein	1.2	**1.5**	1.3
gluc2	TAY18212	UP|Q9XEN5 (Q9XEN5) Beta-1 3-glucanase	1.4	**1.5**	1.2
HVSMEh0095G24f	BE454722	similar to UP|LU1A_LYCPN (O04973) 2-isopropylmalate synthase A	1.1	**1.5**	**2.0**
HV_CEb0006G24f	BE215306	UP|O82072 (O82072) Phosphoenolpyruvate carboxylase	1.5	**1.5**	**1.3**
SFR011.B06F990706	BE438124	homologue to UP|Q8GTK9 (Q8GTK9) Putative asparaginyl-tRNA synthetase chloroplast/mitochondrial	1.4	**1.4**	1.3
wir1b	WHTWIR1PR	UP|WIRB_WHEAT (Q01481) WIR1B protein	1.2	**1.4**	1.3
HV_CEb0003J11f	BE214483	UP|Q43765 (Q43765) Chitinase (EC 3.2.1.14 EC 3.2.1.14)	1.2	**1.4**	1.3
HV_CEb0003K12f	BE214507	UP|P93180 (P93180) Pathogenesis-related protein 4 precursor	0.7	**1.4**	**1.3**
wir1c	TARNAWIR1	UP|Q41581 (Q41581) WIR1 protein	1.0	**1.4**	1.3
HV_CEb0009B05f	BE216003	homologue to UP|Q9FRT5 (Q9FRT5) Monosaccharide transporter 3	1.5	**1.4**	1.0
HVSMEg0017G18f	BE231181	homologue to UP|Q75HE6 (Q75HE6) Putative methylenetetrahydrofolate reductase	1.0	**1.3**	1.1

[a] : clones that have not given the same blast result as in the database.

Table 12: Differentially expressed genes in the field trial 72 hrs after treatment with either 2,4-D, cinidon-ethyl (C), and tribenuron-methyl (T). Intensity ratios of genes determined to be differentially expressed by SAM analysis are in bold type. The positive values indicate gene induction and negative values indicate gene repression.

Gene ID	Accession number	Putative function	2,4-D	C	T
HV_CEb0009E12f	AV931424	UP|THN7_HORVU (Q42838) Thionin BTH7 precursor	**1.6**	1.4	1.5
HVSMEh0095K23f	BE454522	homologue to emb|X00755.1|OSRRN17S Rice gene for 17S ribosomal RNA	**1.4**	-1.0	1.4
HVSMEg0005E01f	BG343260	UP|SYE_HORVU (Q43768) Glutamyl-tRNA synthetase (GluRS)	**1.3**	1.1	1.4
HVSMEg0008B09f	BG344201	homologue to UP|Q6Z2M3 (Q6Z2M3) Putative phosphatidate cytidylyltransferase domain-containing protein	**1.3**	1.1	1.3
HVSMEg0012F22f	BE060461	homologue to UP|Q6ZHC3 (Q6ZHC3) Putative aspartate-tRNA ligase	1.1	1.2	**1.6**
HVSMEg0002M03f	AW982294	homologue to UP|Q6H4P7 (Q6H4P7) Putative leucyl-tRNA synthetase	1.1	1.1	**1.7**
HVSMEg0010A06f	AW983267	similar to UP|Q6PW76 (Q6PW76) Delta-1-pyrroline-5-carboxylate synthetase (EC 2.7.2.11 EC 1.2.1.41)	1.1	1.0	**1.5**
HVSMEh0095G24f	BE454722	similar to UP|LU1A_LYCPN (O04973) 2-isopropylmalate synthase A	1.1	1.6	**3.6**
HVSMEg0002O09f	AW982323	homologue to UP|Q9LKM0 (Q9LKM0) Nucleoside diphosphate kinase (EC 2.7.4.6)	1.0	1.0	**1.6**
HV_CEb0010L20f	BE216529	UP|PR1_HORVU (Q05968) Pathogenesis-related protein 1 precursor	1.0	**1.6**	1.4
HVSMEh0088K24f	BE195244	UP|Q42839 (Q42839) Chitinase (EC 3.2.1.14)	1.0	**1.7**	1.4
HV_CEb0003A01f	BE214283	UP|Q43764 (Q43764) Chitinase (EC 3.2.1.14)	1.0	**1.7**	**1.7**
HVSMEg0002O22f[a]	BE231062	homologue to UP|Q9LKM0 (Q9LKM0) Nucleoside diphosphate kinase (EC 2.7.4.6)	1.0	1.0	**1.6**
HVSMEg0005K03f	BG343392	homologue to UP|Q8RZF3 (Q8RZF3) Putative ketol-acid reductoisomerase	1.0	1.0	**1.8**
wir232	TATHAU	UP|Q94F70 (Q94F70) Thaumatin-like protein	1.0	**1.5**	1.2
HV_CEb0009E24f	BE216070	UP|FER_WHEAT (P00228) Ferredoxin chloroplast precursor	-1.0	-1.1	**1.5**
HVSMEh0101A21f	BE602672	UP|CHI1_HORVU (P11955) 26 kDa endochitinase 1 precursor (EC 3.2.1.14)	-1.1	**1.9**	**1.8**
wci4	TAU32428	UP|Q41520 (Q41520) Lipoxygenase (Fragment) (EC 1.13.11.12)	-1.1	-1.1	**1.7**
pox381	X56011	UP|Q43212 (Q43212) Peroxidase precursor (EC 1.11.1.7)	-1.1	**1.9**	**1.7**
HV_CEb0024H02f	BE559387	UP|Q43764 (Q43764) Chitinase (EC 3.2.1.14)	-1.1	**1.7**	1.5
wci2	TC139173	homologue to UP|Q42847 (Q42847) Lipoxygenase 2 (EC 1.13.11.12)	-1.2	-1.1	**1.8**
HV_CEb0021P01f	BE519980	UP|Q40068 (Q40068) Peroxidase (EC 1.11.1.7)	-1.2	**1.7**	1.4
HV_CEb0003B05f[a]	BE214500	homologue to UP|WIRA_WHEAT (Q01482) WIR1A protein	-1.2	**1.5**	1.3

[a] : clones that have not given the same blast result as in the database.

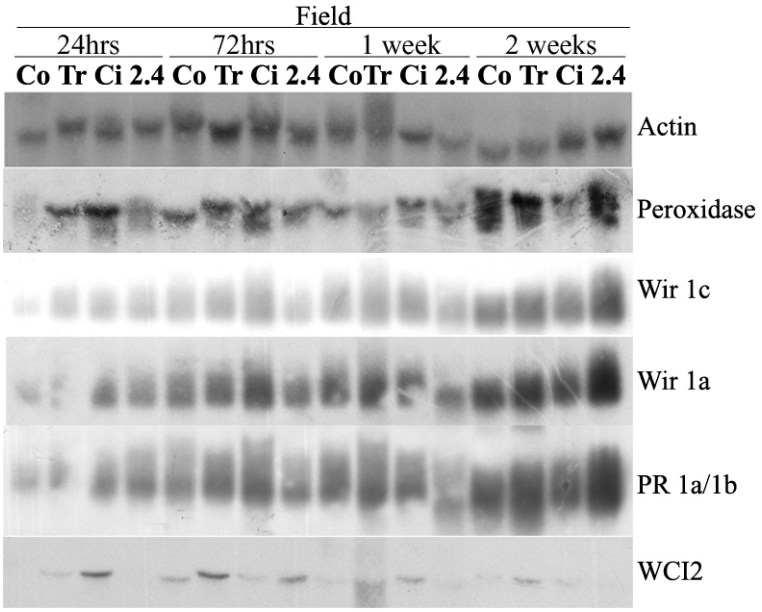


Figure 21: RNA blot analysis showing differential expression 24h, 72 hrs, one and two weeks after herbicide treatment of wheat in the field trial. Co: control, Tr: tribenuron-methyl, Ci: cinidon-ethyl, 2.4: 2,4-D. Seven labelled probes were used: actin gene, as quality control of the blots, peroxidase gene (pox381), WIR 1c gene, WIR 1a gene, pathogenesis related PR 1a/1b gene (HV_CEb0006J08f) and WCI2 gene. Similar expression levels of the genes are visible between the controls and treated plants for all time points, except for the WCI2 gene.

synthase gene and two kinase genes were down-regulated after two weeks (supplemental Table 3).

In the field, the treatment with this inhibitor of the ALS resulted in the up-regulation of the isopropylmalate synthase gene (involved in branched-chain amino acid synthesis) which was two-fold up-regulated after 24 hrs and 3.6-fold after 72 hrs (Table 11 and 12, Figure 19B). Two chitinase genes, two lipoxygenase genes and a peroxidase gene were nearly two-fold induced after 72 hrs. The aspartate tRNA ligase and the leucyl tRNA synthetase, and the ketol-acid reductoisomerase gene were also showing a slightly higher expression at this time point.

5.5 Discussion

We have shown that herbicides trigger the expression of wheat genes from different metabolic pathways early after treatment. Under controlled environmental conditions in the phytotron, 2,4-D and cinidon-ethyl induced genes probably involved in their detoxification, whereas tribenuron-methyl treatment resulted only in a later expression of defence-related genes. In the field trial, results similar to the controlled condition experiment were obtained after cinidon-ethyl treatment but no effect was detected after 2,4-D treatment. In contrast to the controlled conditions, field treatment of tribenuron-methyl resulted in the induction of a gene from the branched-chain amino acid biosynthesis pathway.

5.5.1 Effect of 2,4-D on wheat gene expression

In our studies, 2,4-D treatment of the resistant wheat induced after 24hrs the auxin-induced protein IAA1 gene which is involved in repression of early auxin response genes at low auxin concentrations. This indicates that resistance might not only occur via detoxification of the herbicide but also that wheat protects itself against aberrant developmental auxin-induced responses (Grossmann and Scheltrup, 1998; Grossmann and Kwiatkowski, 2000).

Table 13: Differentially expressed genes in the field trial one week after treatment with either 2,4-D, cinidon-ethyl (C), and tribenuron-methyl (T). Intensity ratios of genes determined to be differentially expressed by SAM analysis are in bold type. The positive values indicate gene induction and negative values indicate gene repression.

Gene ID	Accession number	Putative function	2,4-D	C	T
HV_CEb0023H18f	BE559296	similar to UP|Q42810 (Q42810) GmCK2p (EC 2.7.1.32) choline kinase	1.5	**-1.7**	1.3
HVSMEg0001K11f	BG343000	similar to PIR|T05536 acid phosphatase (EC 3.1.3.2) - Arabidopsis thaliana	1.4	**-2.1**	1.0
HV_CEb0020E03f	BE558748	homologue to UP|UBA2_WHEAT (P31251) Ubiquitin-activating enzyme E1 2	1.2	**-1.5**	1.2
HVSMEg0002E08f	AW982203	homologue to UP|Q8SA22 (Q8SA22) Putative pyruvate kinase	1.1	**-1.6**	1.1
HV_CEb0011G08f	BE216736	similar to UP|O23637 (O23637) Argininosuccinate lyase (EC 4.3.2.1)	1.1	**-1.5**	1.1
HV_CEb0010N04f	BE216561	homologue to UP|Q41328 (Q41328) Pto kinase interactor 1	1.0	**-1.6**	-1.4
HV_CEb0021G05f	BE216563	homologue to GP|17981573 kinase R-like protein {Triticum aestivum}	-1.1	**-1.6**	-1.0
HV_CEb0010E08f	BE216352	similar to UP|Q7XHB3 (Q7XHB3) Putative peroxidase	-1.1	**-1.5**	-2.1
HV_CEb0010N06f	BE216563	homologue to GP|17981573 kinase R-like protein {Triticum aestivum}	-1.1	**-1.7**	-1.4
HVSMEg0004L06f	AW982972	similar to UP|Q8S505 (Q8S505) Acid phosphatase	-1.2	**-1.8**	-1.9
HVSMEg0005A08f	BF623345	UP|THN7_HORVU (Q42838) Thionin BTH7 precursor	-1.3	**-1.5**	1.1
HVSMEg0006G05f	BG343558	UP|INO1_HORVU (O65195) Inositol-3-phosphate synthase	-1.3	**-1.5**	-2.1
HV_CEb0010N22f	BE216579	similar to UP|Q6YUU3 (Q6YUU3) Putative leucine-rich repeat transmembrane protein kinase	-1.3	**-1.6**	-1.5
HV_CEb0016L17f	BE519519	similar to UP|Q7F169 (Q7F169) S-receptor kinase PK3-like protein	-1.4	**-1.5**	-1.5
HV_CEb0023F16f	BE559272	homologue to UP|STAD_ORYSA (Q40731) Acyl-[acyl-carrier-protein] desaturase chloroplast precursor (Stearoyl-ACP desaturase)	-1.4	**-1.5**	-1.3
HVSMEg0002E11f	AW982206	similar to UP|Q6Z7L1 (Q6Z7L1) Putative dnaK-type molecular chaperone	-1.5	**-1.7**	-1.5
HVSMEg0003D05f	AW982411	weakly similar to UP|Q75IK0 (Q75IK0) Putative o-methyltransferase ZRP4	**-1.6**	-1.4	-1.6
HV_CEb0010C13f	BE216310	similar to UP|Q43700 (Q43700) Heat shock factor	**-1.8**	-1.3	-1.3
HV_CEb0017H19f	BE558391	similar to UP|Q94IP1 (Q94IP1) Cinnamic acid 4-hydroxylase (EC 1.14.13.11)	**-1.9**	-1.2	-2.0
gluc2	TAY18212	UP|E13B_HORVU (P15737) Glucan endo-1 3-beta-glucosidase GII precursor	**-2.0**	1.1	-1.3
HV_CEb0015D24f	BE558211	homologue to UP|Q75GF5 (Q75GF5) Putative general negative regulator of transcription subunit 3'-partial (Fragment)	-2.0	**-1.4**	-2.0
HV_CEb0003K16f	BE214511	UP|Q7XJ80 (Q7XJ80) Cytosolic heat shock protein 90	**-2.1**	**-1.5**	-2.4
HV_CEb0006G24f	BE215306	UP|O82072 (O82072) Phosphoenolpyruvate carboxylase	**-2.2**	-1.2	-2.9
HVSMEg0013D03f	BE060737	homologue to UP|Q6QWQ3 (Q6QWQ3) Fructose 1 6-bisphosphate aldolase (EC 4.1.2.13)	**-2.5**	-1.0	-1.9
HV_CEb0009I05f	AJ251298	homologue to UP|Q8H413 (Q8H413) Spermidine synthase 1	**-2.6**	-1.4	-2.2
HV_CEb0009B05f	BE216003	homologue to UP|Q9FRT5 (Q9FRT5) Monosaccharide transporter 3	**-2.7**	**-1.6**	-2.0
HV_CEb0006C18f	BE215212	UP|Q43475 (Q43475) SNF1-related protein kinase (Fragment)	**-2.7**	-1.3	-2.8
HVSMEh0090J03f	BE195825	UP|METK_HORVU (P50299) S-adenosylmethionine synthetase 1	**-2.9**	-1.1	-3.4
HV_CEb0003P20f	BE214619	UP|CHS1_HORVU (P26018) Chalcone synthase 1	**-2.9**	-1.3	-3.3
HV_CEb0004G09f	TC140105	similar to UP|Q8GT52 (Q8GT52) Hexose transporter	**-4.7**	**-2.0**	-3.5
HV_CEb0001H04f	unknown	unknown	**-4.7**	-1.0	-3.2

In sensitive species, auxin-like herbicide treatment is followed by an increase of 1-aminocyclopropane-1-carboxylate (ACC) synthase gene expression and then by ethylene production (Grossmann, 2000b). The S-adenosylmethionine synthetase gene, involved in ethylene biosynthesis, was slightly up-regulated in our study, suggesting that 2,4-D could trigger similar initial responses in wheat as in sensitive species. However, the ACC synthase gene and the ethylene-forming-enzyme-like dioxygenase gene were not induced in wheat indicating that the resistance might involve a process preventing ethylene formation, downstream of the S-adenosylmethionine synthetase step.

The up-regulation of other genes revealed more on the possible detoxification of 2,4-D in wheat. The cytochrome P450 86A1 gene that encodes a fatty acid omega-hydroxylase with broad substrate range (Benveniste et al., 1998) showed up-regulation after 24 hrs. This enzyme could be involved in the detoxification process by aryl-hydroxylation of 2,4-D and other auxin-like herbicides (phase I) (Cabello-Hurtado et al., 1998; Robineau et al., 1998). Auxin-like herbicides are neither C12 nor C18 fatty acids, but their three-dimensional structures are similar to fatty acids, the usual substrates of such enzymes (Zimmerlin and Durst, 1990).

Treatment with 2,4-D also resulted in the increased expression of several phenylpropanoid pathway genes (PAL, C4H, COMT). C4H expression is induced after wounding or treatment with xenobiotics (Batard et al., 1997), and the encoded enzyme could be involved in the hydroxylation of 2,4-D to form 4-OH-2,5D (Baerg et al., 1996; Schuler and Werck-Reichhart, 2003). The herbicide can be incorporated intact or as a monohydroxylated form into the lignin network of the cell wall in wheat (Scheel and Sandermann, 1981). The induction of the COMT and chalcone synthase genes suggests that hydroxylated or intact 2,4-D can be methylated and form a monolignol or a flavonoid.

5.5.2 Changes in wheat expression induced by cinidon-ethyl

Peroxidase genes were the most highly induced genes after cinidon-ethyl treatment. They are involved in the final steps of lignin and suberin biosynthesis but are also known to oxidise pesticides (Katagi and Mikami, 2000). Therefore, they could be involved in one of the first steps of the detoxification of cinidon-ethyl to form a hydroxylated product that could be further modified by phenylpropanoid pathway enzymes or be conjugated for subsequent metabolisation (Devine et al., 1993a). This role is usually played by a phytochrome P450 monoxygenase, an enzyme that seems to be involved in the metabolism of cinidon-ethyl (K. Grossmann, BASF, Germany, personal communication). The C4H (cytochrome P450 73A) gene was the only cytochrome P450 gene of our microarray that showed differential expression 24 hrs after treatment, although with less statistical confidence (FDR 9%) than the other results (FDR 4%). C4H is known to be induced in response to chemical treatments (naphtalic anhydride in maize (Persans et al., 2001) or phenobarbital in Jerusalem artichoke (Batard et al., 1997)). Peroxidases are less substrate-specific than glutathione S-transferase (GST) but they can conjugate glutathione to unsaturated phenylpropanoid or secondary compounds (Dean and Devarenne, 1997). However, our data indicate that, upon cinidon-ethyl treatment, peroxidase genes were stronger activated than GST and C4H genes which could indicate that peroxidases are more important for cinidon-ethyl metabolisation.

Other cellular responses might also occur after the herbicide treatment. In the presence of light and oxygen, the herbicide induces proto-IX accumulation which generates reactive oxygen species (ROS) that are known to trigger ethylene synthesis (Wang et al., 2002). The inhibition of Protox enzyme in *Arabidopsis thaliana* expressing Protox anti-sense RNA induces systemic acquired resistance (Molina et al., 1999). Our study revealed that cinidon-ethyl can induce the ethylene signalling pathway, as suggested by the up-regulation of the ethylene-forming-enzyme-like dioxygenase gene. Ethylene and ROS can trigger defence-

related genes (WIR genes, 1,3-endoglucanase genes and chitinase genes) and these genes were over-expressed 24 hrs and one week after treatment but not after 72 hrs. Peroxidases are involved in defence responses and the over-expression of the peroxidase genes could be due to ethylene. Possibly, the wheat plant is protected against pathogen attack by the activation of defence response during the early detoxification process (after 24 hrs) and during a secondary response phase (after one week).

5.*5.3 Effect of tribenuron-methyl on wheat gene expression*

In contrast to the first two herbicides, application of tribenuron-methyl under controlled growth conditions did not result in changes in gene expression 24 hrs after treatment. This could be explained by the fact that the half-life of this type of herbicide is only of 2-3 hrs in wheat and other cereals. In these crops, no sulfonylurea (intact or degraded) is accumulated in straw or grain extracts because of a fast detoxification process (Sweetser et al., 1982; Brown, 1990). A rapid uptake of this molecule might explain the fast triggering of the detoxification process as compared to 2,4-D or cinidon-ethyl (Brown, 1990; Sterling and Hall, 1997; Grossmann and Schiffer, 1999).

However, after one week, treated plants showed induction of the WIR1a, 1b, 1c and thaumatin-like genes as well as the PR1, 1a/1b and 4 genes, involved in defence reaction. We speculate that this induction is due to some metabolites of tribenuron-methyl, e.g. the bensizothiazole dioxide derivatives that can induce systemic acquired resistance (Roberts, 1998b; Davies and Caseley, 1999; Yoshioka et al., 2001). Another type of tribenuron-methyl derivatives, benzenesulfonamide, is used as herbicide safener that can facilitate xenobiotic detoxification through regulation of gene expression (DeVeylder et al., 1997).

5.5.4 Impact of the herbicides in the field

In comparison to the plants grown under controlled conditions, plants grown in the field showed fewer changes in gene expression after treatment with the herbicides. In particular, 2,4-D treatment in the field did not induce the genes possibly involved in detoxification, but triggered down-regulation of genes of the phenylpropanoid pathway one week after the treatment. These discrepancies in relation to the controlled environment are currently not understood.

In contrast to 2,4-D, cinidon-ethyl treatment induced similar changes in gene expression in both environments. Peroxidase and defence-related genes were induced, albeit at a lower level compared to the plants grown under controlled conditions. However, up-regulation of this set of genes was prolonged in the field up to 72 hrs after treatment whereas it was only observed after 24 hrs under controlled conditions. In untreated plants, defence-related genes are already expressed at high levels probably because of several stresses occurring in the field (Rizhsky et al., 2002; Voelckel and Baldwin, 2004). Thus, in treated plants in the field, a smaller induction might be sufficient to allow the detoxification of xenobiotics.

Finally, in contrast to the experiment in controlled conditions, treatment with tribenuron-methyl induced a gene from the branched-chain amino acid biosynthesis pathway in the field. The isopropylmalate synthase gene, which is under the control of leucine level, was up-regulated after 24 and 72 hrs. This result suggests that the wheat detoxification system is less efficient in the field than in controlled conditions and that the plants react to the inhibition of acetolactate synthase by the herbicide through the induction of the isopropylmalate synthase gene, possibly because of a reduced feedback inhibition by leucine.

5.6 Conclusion

Our study has given an overview of the changes in gene expression induced by herbicide treatments of crop plants. The results provide new insight on the effects of these molecules on wheat metabolism, and possibly on the detoxification processes of these three xenobiotics. The microarray technique has revealed that different expression patterns are triggered in the plant depending on the herbicide used. We have also observed different responses depending on growth conditions. Treatment with herbicides in controlled conditions rarely altered the expression of genes from primary metabolism, indicating that herbicides trigger very specific responses. Metabolisation appeared to be the major way for resistance in wheat against the xenobiotics studied here. These detoxification processes are very rapidly established as gene expression profiles were significantly different between the first time point (24 hrs) and the following ones, where fewer genes were differentially expressed. The expression patterns after treatment with tribenuron-methyl under controlled conditions reflected a fast detoxification system for this kind of xenobiotic whose half-life is just two hours in wheat (Koeppe et al., 1997). The ability of wheat to detoxify the xenobiotics over time seems to be more sensitive to environmental conditions for tribenuron-methyl than for the two other compounds. Finally, the induction of defence-related genes after cinidon-ethyl and tribenuron-methyl treatment suggests that these compounds could trigger a systemic acquired resistance-like response and therefore might be beneficial for the plants to respond against pathogen attack. Whether treated plants are indeed more resistant to pathogens needs to be investigated further.

5.7 Acknowledgments

We would like to thank Geri Herren for his technical assistance, Beatrice Senger and Dr. Gabriele Schachermayr for their help with field experiments. We thank Dr. Célia Baroux and Dr. Nabila Yahiaoui for critical reading of the manuscript. This work was supported by the Swiss Federal Office of Public Health, contract no. 01.000004 (Reg. 2.26.02.-14).

VI. General discussion

The use of agrochemicals has become essential for the protection of crop plants. However, although substantial equivalence has to be demonstrated for GMOs in relation to the plant of origin, this criterion is not studied after xenobiotic treatment of a crop. Our transcriptome study on wheat after herbicide and fungicide treatments was initiated to analyse the putative impact of these compounds at the gene expression level.

Transcriptome studies open new possibilities to study genes that share regulatory patterns and to detect interconnected pathways. Studies on the model plant *Arabidopsis thaliana* have resulted in new insights in plant metabolism, development and response to different stresses. However, results obtained from this dicot plant are not always applicable to plants of agronomic interest such as grasses. Wheat belongs to this family and its study for the discovery of genes of agricultural importance (involved in quality, resistance) is hampered by the size and complexity of its hexaploid genome. The possibility to use genetically collinear species such as rice, barley or diploid wheat has allowed to isolate two disease resistance genes from wheat (Feuillet et al., 2003; Yahiaoui et al., 2004). Here, we have used the high similarity of coding sequence between barley and wheat for gene expression studies.

These studies have revealed that wheat is reacting differentially depending on the compound studied. The two fungicides azoxystrobin and fenpropimorph induced defence-related gene expression, in a pattern similar to the one observed after BTH treatment, although only this last product was known to induce such expression changes in wheat (Görlach et al., 1996). Furthermore, these effects were still visible two weeks after the treatments in the greenhouse. In contrast to these lasting effects, the impact of the herbicide 2,4D on wheat gene expression was very transient and only few genes were differentially expressed 24hrs after treatment. The cinidon-ethyl and tribenuron-methyl herbicides induced the expression of defence-related genes after one week in a manner that could be similar to the enhancement of the SAR after BTH treatment, albeit at a lower level and for a short period. These results have demonstrated

that agrochemicals have a demonstrable effect on the plants they are protecting concerning gene regulation.

Interestingly, the results after fungicide treatments were different when the plants were grown in the field. The specific induction of defence-related genes, observed in the greenhouse trial, was prevented by the natural/constitutive high level of expression of these genes in the field. This difference of results between the two growth conditions implies that the observed higher resistance of wheat against pathogen attack after BTH treatment could not only be due to the gene regulation but could also involve other mechanisms such as protein activation. The expression profiles of field grown plants have revealed higher amounts of defence-related transcripts compared to greenhouse grown plants even in the absence of chemical treatments. Moreover, one PR14 gene showed a 92-fold higher transcript level in the field than in the greenhouse. This putative lipid transfer protein gene could be involved in the signalling of defence response such as other LTP genes, the Arabidopsis *DIR1*, the maize *IIG1* and the tobacco *LTP1* (Huang et al., 1998; Maldonado et al., 2002; Buhot et al., 2004). Further studies of this putative LTP gene should improve our understanding of the defence signalling pathway in cereal.

Treatments of wheat with the chosen xenobiotics have resulted in expression of very specific sets of genes in the flag leaves. These genes have no known impact on grain filling but are involved in defence response. In wheat, 80% of the allergens belong to the prolamin family which contains insoluble gliadins and glutenins (Battais et al., 2003). However, soluble pathogenesis-related proteins and lipid transfer proteins have also been categorised as allergenic compounds (Yamashita et al., 2002; Salcedo et al., 2004). Furthermore, these proteins are often resistant to proteolysis and heat and are not destroyed during processing of the flour (Simonato et al., 2001; Asensio et al., 2004). Therefore, it seems that application of the xenobiotic compounds could increase the potential allergenic status of wheat by

increasing the level of defence-related gene transcription. However, our analyses were carried out on the flag leaf and not on the spike itself. In addition, our study was only focusing on one level of plant response: the gene expression. Further studies determining the effective presence of proteins, sugars or products from the secondary metabolism resulting from the activation of these genes have to confirm this first assessment of the impacts of xenobiotic treatments on wheat metabolism.

Future prospects

The studies described in this thesis have provided new insights on the impact of six agrochemicals on wheat gene expression. However, mRNA amounts could not always be correlated to an effect on metabolism because of the multiple regulation levels occurring after transcription (RNA translation, post-translational modifications and turn-over). Nevertheless, the high induction levels occurring after some of the treatments (up to 60-fold induction) suggest that the newly made mRNA is translated. Transcription is costly for the plant and keeping such amounts of mRNA without translating them is unlikely. Therefore, complementary studies at the protein level should be performed to confirm that these agrochemicals have an effective impact at the wheat protein level. Furthermore, analysis of the metabolome to measure the amount of sugars, amino acids, phenylpropanoids or other end-products should give a final overview about the pathways which are the most affected by xenobiotics.

Further work on the putative lipid transfer protein gene and its corresponding protein have to be performed in barley and wheat in order to define if this protein has the same properties as its maize homologue IIG1 which is proposed to be a regulator of ethylene biosynthesis. The study of mutant plants lacking this gene will reveal whether there is any change in pathogen response because of a reduced defence signalling. As ethylene is involved in many pathways

in the plant, other signalling routes might counteract the effect of such mutation. Over-expression of this gene is a second strategy to study its putative function. Study of its transcription profile in different conditions (after BTH treatment and pathogen attacks) could also allow to determine which stimulus can trigger or repress its expression and to find out if this gene is effectively involved in defence signalling in wheat and barley.

VII. References

VII. References

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VII. Appendixes

Appendix 8.1

Blast analyses for the genes present on the barley microarrays.

Gene ID	Gene accession	Putative function Barley database	% Identity	Barley database hit	Wheat database blast	Putative function Wheat database	Wheat database hit
645	HVU234400	UP|Q8H1L9 (Q8H1L9) Actin	100	1.9e-152	TC203563	UP|Q8H1L9 (Q8H1L9) Actin	1.4e-153
Clemson6-36	AJ010093	similar to UP|O82668 (O82668) MAP3K beta 1 protein kinase (EC 2.7.1.37)	72.77	6.1e-67	TC194880	similar to UP|O82668 (O82668) MAP3K beta 1 protein kinase (EC 2.7.1.37)	2.7e-61
GapC	At3g04120	glyceraldehyde-3-phosphate dehydrogenase C subunit (GapC)	100	2.9e-147	TC205817	UP|G3PX_HORVU (P26517) Glyceraldehyde-3-phosphate dehydrogenase cytosolic	1.3e-146
gluc2	TAY18212	UP|E13B_HORVU (P15737) Glucan endo-1 3-beta-glucosidase GII precursor	100	9.3e-223	TC225609	UP|Q9XEN5 (Q9XEN5) Beta-1 3-glucanase	7.1e-120
H2	AU124075	Dihydrofolate reductase [Homo sapiens]					
H4	CB962800	GB|AAN84548 beta globin chain variant {Homo sapiens;}					
H5	NM_002507	UP|TR16_HUMAN (P08138) Tumor necrosis factor receptor superfamily member 16 precursor					
HV_CEa0002M20f	unknown	no hit			no hit	no hit	
HV_CEa0016G14f	unknown	no hit			no hit	no hit	
HV_CEa0016M13f	BF267043	UP|Q9M6N6 (Q9M6N6) RNase S-like protein	100	2.4e-113	TC210470	homologue to UP|Q9M6N6 (Q9M6N6) RNase S-like protein	7.9e-95
HV_CEa0017C20f	BF267177	similar to GB|AAB86804 pyruvate dehydrogenase E1 beta subunit {Arabidopsis thaliana;} (EC 1.2.4.1)	84.15	3.7e-142	TC221904	similar to GB|AAB86804 pyruvate dehydrogenase E1 beta subunit {Arabidopsis thaliana;} (EC 1.2.4.1)	8.6e-125
HV_CEa0017H10f	BF267281	homologue to UP|Q7XB60 (Q7XB60) Putative zeta-carotene desaturase	93.76	8.8e-114	CA642533	homologue to GP|10185572 zeta-carotene desaturase precursor {Oryza sativa}	1.4e-78
HV_CEa0017L24f	unknown	no hit			no hit	no hit	
HV_CEb0001A10f	unknown	no hit			no hit	no hit	
HV_CEb0001B15f	BE213724	homologue to UP|Q8LNW1 (Q8LNW1) Putative transcription factor	90.94	3.6e-132	TC188729	homologue to UP|Q84VF8 (Q84VF8) Putative transcription factor BTF3	1.9e-116
HV_CEb0001E21f	BE213797	similar to UP|O23887 (O23887) Aldehyde oxidase (EC 1.2.3.1)	80.91	1.3e-124	TC193326	similar to UP|O23887 (O23887) Aldehyde oxidase (EC 1.2.3.1 EC 1.2.3.1)	e-112
HV_CEb0001F01f	BE213800	weakly similar to SP|P93149 Cytochrome P450 93B1 (EC 1.14.-.-) ((2S)-flavanone 2-hydroxylase) (Licodione synthase)	49.37	1.3e-202	CA660516	weakly similar to SP|P93149 Cytochrome P450 93B1 (EC 1.14.-.-) ((2S)-flavanone 2-hydroxylase) (Licodione synthase)	3.5e-52
HV_CEb0001G11f	BE213834	homologue to UP|Q93VK6 (Q93VK6) 3-phosphoshikimate 1-carboxyvinyltransferase	95.06	1.7e-110	TC192225	homologue to UP|O24566 (O24566) 3-phosphoshikimate 1-carboxyvinyltransferase (Epsp-synthase) (Fragment) (EC 2.5.1.19)	7.3e-98
HV_CEb0001H04f	unknown	no hit			no hit	no hit	
HV_CEb0001H14f	unknown	no hit			no hit	no hit	
HV_CEb0001H21f	BE213865	similar to UP|Q9M7S1 (Q9M7S1) 4-coumarate--CoA ligase 4CL3 (EC 6.2.1.12)	87.55	1.5e-127	TC221950	homologue to UP|Q9M7S1 (Q9M7S1) 4-coumarate--CoA ligase 4CL3 (EC 6.2.1.12)	1.6e-113
HV_CEb0001J06f	BE213893	UP|Q40028 (Q40028) Beta-ketoacyl-ACP synthase (EC 2.3.1.41)	100	1.7e-99	CA603276	homologue to GP|23954355 metallothioneine type2 {Hordeum vulgare }	7.9e-72
HV_CEb0001J24f	BE213910	similar to UP|Q9AQZ5 (Q9AQZ5) Putative heat-shock protein	83.96	2.9e-142	TC206116	similar to UP|Q9AQZ5 (Q9AQZ5) Putative heat-shock protein	2.9e-134
HV_CEb0001K11f	BE213920	similar to UP|Q6ZL26 (Q6ZL26) Mitogen activated protein kinase kinase	87.41	2.1e-151	TC195968	similar to UP|Q6ZL26 (Q6ZL26) Mitogen activated protein kinase kinase	1.1e-39

Gene ID	Gene accession	Putative function Barley database	% Identity	Barley database hit	Wheat database blast	Putative function Wheat database	Wheat database hit
HV_CEb0001P03f	BE214026	UP|FER_WHEAT (P00228) Ferredoxin chloroplast precursor	99.3	6.0e-102	TC204556	UP|FER_WHEAT (P00228) Ferredoxin chloroplast precursor	5.8e-92
HV_CEb0002C16f	BE214080	UP|ENPL_HORVU (P36183) Endoplasmin homolog precursor (GRP94 homolog)	100	9.8e-108	BG909592	homologue to SP|P36183 Endoplasmin homolog precursor (GRP94 homolog). [Barley] {Hordeum vulgare}	3.00e-70
HV_CEb0002D20f	BE214100	similar to UP|Q6ZCF0 (Q6ZCF0) Putative gamma-aminobutyrate transaminase subunit isozyme 3	81.89	1.3e-161	TC192495	similar to UP|Q6ZCF0 (Q6ZCF0) Putative gamma-aminobutyrate transaminase subunit isozyme 3	7.8e-150
HV_CEb0002E02f	BE214105	homologue to UP|Q6ZEZ1 (Q6ZEZ1) Putative ribose-5-phosphate isomerase	91.6	2.1e-125	TC211762	homologue to UP|Q6ZEZ2 (Q6ZEZ2) Putative ribose-5-phosphate isomerase	8.1e-100
HV_CEb0002G14f	BE214142	homologue to UP|Q84L52 (Q84L52) Metallothionein-like protein type 3	98.39	8.4e-108	TC204448	homologue to UP|Q84L52 (Q84L52) Metallothionein-like protein type 3	1.3e-76
HV_CEb0002G16f	BE214143	UP|Q40027 (Q40027) Beta-ketoacyl-ACP synthase (EC 2.3.1.41)	100	1.2e-124	TC223532	homologue to UP|Q40027 (Q40027) Beta-ketoacyl-ACP synthase (EC 2.3.1.41)	2.9e-96
HV_CEb0002J11f	BE214187	UP|Q7PCC5 (Q7PCC5) Putative cysteine protease precursor	100	1.4e-102	TC217510	homologue to UP|CYS1_MAIZE (Q10716) Cysteine proteinase 1 precursor (EC 3.4.22.-)	2.0e-30
HV_CEb0002K12f	unknown	no hit			no hit	no hit	
HV_CEb0002N07f	BE214245	homologue to UP|Q8LPA2 (Q8LPA2) Chloride channel	91.81	1.5e-37	CA735869	similar to GP|21321024 chloride channel {Oryza sativa (japonica cultivar-group)}	5.1e-58
HV_CEb0002P23f	BE214281	homologue to UP|UBC5_ARATH (P42749) Ubiquitin-conjugating enzyme E2-21 kDa 2 (Ubiquitin-protein ligase 5)	92.36	4.1e-56	TC203826	homologue to UP|UBC4_ARATH (P42748) Ubiquitin-conjugating enzyme E2-21 kDa 1 (Ubiquitin-protein ligase 4)	2.7e-100
HV_CEb0003A01f	BE214283	UP|Q43764 (Q43764) Chitinase (EC 3.2.1.14)	100	1.0e-150	AB029934	UP|Q8W429 (Q8W429) Chitinase 1	6.8e-114
HV_CEb0003A03f	BE214285	similar to GP|13897320 somatic embryogenesis receptor-like kinase 2 {Zea mays}	88.89	2.2e-27	BE586127	weakly similar to UP|P93529 (P93529) Sorghum bicolor leucine-rich repeat-containing extracellular glycoprotein precursor	e-156
HV_CEb0003A20f	BE214301	similar to UP|Q9SLY9 (Q9SLY9) Cysteine protease component of protease-inhibitor complex	84.07	9.4e-85	TC187830	similar to UP|Q9SLY9 (Q9SLY9) Cysteine protease component of protease-inhibitor complex	7.5e-81
HV_CEb0003A24f	BE214305	similar to UP|Q945X2 (Q945X2) Putative glutathione S-transferase OsGSTT1	76.79	1.7e-144	TC221042	similar to UP|Q945X2 (Q945X2) Putative glutathione S-transferase OsGSTT1	1.3e-105
HV_CEb0003B05f[a]	BE214500	homologue to UP|WIRA_WHEAT (Q01482) WIR1A protein	92.31	5.7e-119	TC219253	UP|WIRA_WHEAT (Q01482) WIR1A protein	1.4e-28
HV_CEb0003C09f	BE214334	UP|PGKY_WHEAT (P12783) Phosphoglycerate kinase cytosolic (EC 2.7.2.3)	99.25	1.0e-46	TC190335	UP|PGKH_WHEAT (P12782) Phosphoglycerate kinase chloroplast precursor (EC 2.7.2.3)	2.2e-115
HV_CEb0003D01f	BE214349	UP|O65189 (O65189) Glucan endo-1 3-beta-glucosidase isoenzyme I	100	8.1e-60	AF112967	UP|Q9XEN7 (Q9XEN7) Beta-1 3-glucanase	4.8e-96
HV_CEb0003H19f	BE214449	homologue to UP|Q9ZWJ2 (Q9ZWJ2) Glyoxalase I	92.41	2.6e-72	TC190364	homologue to UP|Q9ZWJ2 (Q9ZWJ2) Glyoxalase I	9.0e-72
HV_CEb0003I04f	BE214511	UP|Q7XJ80 (Q7XJ80) Cytosolic heat shock protein 90	100	1.6e-67	TC190430	homologue to UP|Q9ZRG0 (Q9ZRG0) Heat shock protein 80	1.3e-85
HV_CEb0003I16f	BE214467	UP|Q7XJ80 (Q7XJ80) Cytosolic heat shock protein 90	100	7.1e-62	TC190430	homologue to UP|Q9ZRG0 (Q9ZRG0) Heat shock protein 80	1.3e-85
HV_CEb0003I19f	unknown	no hit			no hit	no hit	
HV_CEb0003I21f	BE214472	similar to UP|Q6ZL26 (Q6ZL26) Mitogen activated protein kinase kinase	87.41	1.5e-32	|TC214450	homologue to UP|Q6ZL26 (Q6ZL26) Mitogen activated protein kinase kinase	1.9e-39
HV_CEb0003J11f	BE214483	UP|Q43765 (Q43765) Chitinase (EC 3.2.1.14)	100	5.6e-110	TC209470	UP|Q8W429 (Q8W429) Chitinase 1	1.4e-92
HV_CEb0003J19f	BE214490	weakly similar to SP|Q43250 Cytochrome P450 71C1 (EC 1.14.-.-). [Maize] {Zea mays}	67.42	6.4e-135	TC224342	UP|Q8S9E9 (Q8S9E9) Cytochrome P450	3.6e-57
HV_CEb0003K02f	BE214497	homologue to UP|Q6ZFI6 (Q6ZFI6) Putative aminotransferase	91.5	2.0e-151	TC203673	homologue to UP|Q6ZFI6 (Q6ZFI6) Putative aminotransferase	8.8e-146
HV_CEb0003K12f	BE214507	UP|P93180 (P93180) Pathogenesis-related protein 4 precursor	100	9.6e-57	TC220170	similar to UP|Q6PWL8 (Q6PWL8) Putative vacuolar defense protein precursor	1.3e-84
HV_CEb0003K16f	BE214511	UP|Q7XJ80 (Q7XJ80) Cytosolic heat shock protein 90	100	1.6e-67	TC190430	homologue to UP|Q9ZRG0 (Q9ZRG0) Heat shock protein 80	1.3e-85
HV_CEb0003K23f	BE214516	UP|Q7XJ26 (Q7XJ26) Iron/ascorbate-dependent oxidoreductase	100	6.2e-115	TC190022	homologue to UP|Q7XJ26 (Q7XJ26) Iron/ascorbate-dependent oxidoreductase	7.5e-119
HV_CEb0003L08f	BE214525	similar to UP|Q93W70 (Q93W70) SERK1 protein precursor (Somatic embryogenesis receptor-like kinase 1 precursor)	87.02	1.7e-27	CA498480	similar to UP|Q93W70 (Q93W70) SERK1 protein precursor (Somatic embryogenesis receptor-like kinase 1 precursor)	2.8e-29

Gene ID	Gene accession	Putative function Barley database	% Identity	Barley database hit	Wheat database blast	Putative function Wheat database	Wheat database hit
HV_CEb0003L15f	BE214532	UP|RCAA_HORVU (Q40073) Ribulose bisphosphate carboxylase/oxygenase activase A chloroplast precursor	99.57	1.1e-153	TC190924	UP|Q9M4V3 (Q9M4V3) Ribulose bisphosphate carboxylase activase B	4.0e-99
HV_CEb0003O02f	BE214583	weakly similar to GP|22535653 putative protein kinase Xa21 receptor type precursor {Oryza sativa (japonica cultivar-group)}	67.63	1.6e-43	CA484845	similar to GP|22535653 putative protein kinase Xa21 receptor type precursor {Oryza sativa (japonica cultivar-group)}	7.2e-41
HV_CEb0003P13f	BE214612	homologue to UP|Q6K1Q8 (Q6K1Q8) Putative phenylalanine ammonia-lyase	90.44	1.6e-150	TC203088	homologue to UP|Q6K1Q8 (Q6K1Q8) Putative phenylalanine ammonia-lyase	1.6e-146
HV_CEb0003P20f	BE214619	UP|CHS1_HORVU (P26018) Chalcone synthase 1 (Naringenin-chalcone synthase 1)	100	3.0e-89	AY286093	UP|CHS2_SECCE (P53415) Chalcone synthase 2 (Naringenin-chalcone synthase 2) (EC 2.3.1.74 EC 2.3.1.74)	1.4e-92
HV_CEb0004B22f	BE214657	UP|ASPR_HORVU (P42210) Phytepsin precursor (Aspartic proteinase) (EC 3.4.23.40)	99.61	1.1e-38	TC187727	homologue to UP|ASPR_HORVU (P42210) Phytepsin precursor (Aspartic proteinase) (EC 3.4.23.40)	3.8e-37
HV_CEb0004C17f	BE214673	similar to GP|12583796 putative cytochrome p450tyr {Oryza sativa}	71.43	1.8e-133	CA629779	UP|Q8S9E8 (Q8S9E8) P450	0.014
HV_CEb0004D06f	BE214683	homologue to UP|Q94IJ4 (Q94IJ4) Somatic embryogenesis receptor-like kinase 2 precursor	90.16	4.5e-29	CA661378	similar to GP|13897310 SERK2 protein {Zea mays}	2.0e-82
HV_CEb0004G09f	TC140105	homologue to UP|Q8GT52 (Q8GT52) Hexose transporter	90.39	6.0e-97	TC197137	similar to UP|Q8GT52 (Q8GT52) Hexose transporter	2.6e-70
HV_CEb0004H24f	BE214757	similar to UP|Q75V57 (Q75V57) Serine/threonine protein kinase SAPK9	88.51	7.3e-122	TC189159	similar to UP|Q75V57 (Q75V57) Serine/threonine protein kinase SAPK9	1.6e-112
HV_CEb0004I05f	BG415346	homologue to UP|Q8S3J5 (Q8S3J5) Ferredoxin precursor	91.39	6.6e-131	TC223230	UP|Q8S3J5 (Q8S3J5) Ferredoxin precursor	2.4e-93
HV_CEb0004J20f	BE214787	similar to GP|13661766 putative cytochrome P450 {Lolium rigidum}	76.57	1.2e-137	TC191895	similar to UP|Q9ATU5 (Q9ATU5) Putative cytochrome P450	7.4e-99
HV_CEb0004K11f[a]	BE214787	similar to GP|13661766 putative cytochrome P450 {Lolium rigidum}	76.57	1.2e-137	TC191895	similar to UP|Q9ATU5 (Q9ATU5) Putative cytochrome P450	7.4e-99
HV_CEb0004M23f	BG299484	UP|Q7XTK5 (Q7XTK5) IAA1 protein	99.14	2.4e-140	TC207223	UP|Q7XTK5 (Q7XTK5) IAA1 protein	5.2e-130
HV_CEb0004N12f	BE214848	weakly similar to UP|P93529 (P93529) Sorghum bicolor leucine-rich repeat-containing extracellular glycoprotein precursor	67.05	4.1e-124	TC226610	weakly similar to UP|P93529 (P93529) Sorghum bicolor leucine-rich repeat-containing extracellular glycoprotein precursor	7.3e-103
HV_CEb0005A11f	BE214887	homologue to UP|Q9AUK8 (Q9AUK8) Putative ferredoxin	92	3.2e-71	TC226615	homologue to UP|Q9AUK8 (Q9AUK8) Putative ferredoxin	8.3e-56
HV_CEb0005E01f	BE214937	similar to UP|MYO3_LYCES (P54928) Inositol-1(or 4)-monophosphatase 3	76.95	1.6e-110	TC195521	similar to UP|MYO3_LYCES (P54928) Inositol-1(or 4)-monophosphatase 3 (IMPase 3) (IMP 3) (Inositol monophosphatase 3)	1.1e-87
HV_CEb0005E11f	unknown	no hit			no hit	no hit	
HV_CEb0005F01f	BE214948	similar to UP|Q6YVV6 (Q6YVV6) Putative serine/threonine-specific protein kinase(Gi|7488195|)	82.7	9.2e-99	TC197696	similar to UP|Q84SG9 (Q84SG9) Serine/threonine kinase receptor-like protein	2.2e-112
HV_CEb0005G20f	BE214980	UP|Q84UD4 (Q84UD4) GAMYB-binding protein	99.79	1.8e-138	TC191498	homologue to UP|Q84UD4 (Q84UD4) GAMYB-binding protein	2.4e-130
HV_CEb0005J09f	BE215029	weakly similar to UP|P93529 (P93529) Sorghum bicolor leucine-rich repeat-containing extracellular glycoprotein precursor	67.05	1.2e-142	TC226610	weakly similar to UP|P93529 (P93529) Sorghum bicolor leucine-rich repeat-containing extracellular glycoprotein precursor	1.1e-117
HV_CEb0005M09f	unknown	no hit			no hit	no hit	
HV_CEb0005M13f[a]	BE215155	homologue to UP|Q6YYB9 (Q6YYB9) Putative tryptophan synthase beta-subunit	95	6.4e-132	TC193090	homologue to UP|Q6YYB9 (Q6YYB9) Putative tryptophan synthase beta-subunit	2.7e-126
HV_CEb0005N17f	BE215115	similar to UP|Q8W5H8 (Q8W5H8) Putative peroxidase	75.81	1.0e-153	TC216170	similar to UP|Q8W5H8 (Q8W5H8) Putative peroxidase	1.1e-85
HV_CEb0006A03f	BE215152	UP|O04876 (O04876) Phenylalanine ammonia-lyase (Fragment) (EC 4.3.1.5)	99.52	1.8e-105	TC205912	UP|PALY_WHEAT (Q43210) Phenylalanine ammonia-lyase (EC 4.3.1.5 EC 4.3.1.5 EC 4.3.1.5)	1.3e-104
HV_CEb0006A07f	BE215155	homologue to UP|Q6YYB9 (Q6YYB9) Putative tryptophan synthase beta-subunit	95	6.4e-132	TC193090	homologue to UP|Q6YYB9 (Q6YYB9) Putative tryptophan synthase beta-subunit	2.7e-126
HV_CEb0006A12f	BE215160	weakly similar to UP|Q84NG8 (Q84NG8) Putative receptor kinase	63.39	1.5e-130	BG274859	similar to GP|25553672 putative protein kinase Xa21 {Oryza sativa (japonica cultivar-group)}	8.5e-19

Gene ID	Gene accession	Putative function Barley database	% Identity	Barley database hit	Wheat database blast	Putative function Wheat database	Wheat database hit
HV_CEb0006B16f	BE215186	similar to UP|Q8S9T6 (Q8S9T6) Putative seryl-tRNA synthetase	87.91	3.9e-105	TC192612	similar to UP|Q8S9T6 (Q8S9T6) Putative seryl-tRNA synthetase	2.7e-100
HV_CEb0006C16f	BE215210	similar to UP|Q7XCK7 (Q7XCK7) Protein kinase-like protein	89.93	4.9e-143	TC192433	similar to UP|Q7XCK7 (Q7XCK7) Protein kinase-like protein	2.8e-129
HV_CEb0006C18f	BE215212	UP|Q43475 (Q43475) SNF1-related protein kinase (Fragment)	99.59	8.7e-66	NP435396	SNF1-related protein kinase [Triticum aestivum]	1.2e-37
HV_CEb0006D17f	BE215231	similar to SP|P30111 Glutathione S-transferase 2 (EC 2.5.1.18) (GST class-phi). [Wheat] {Triticum aestivum}	89.33	5.8e-61	TC206103	homologue to UP|GTH2_WHEAT (P30111) Glutathione S-transferase 2 (GST class-phi) (EC 2.5.1.18)	1.0e-31
HV_CEb0006E10f	BE215247	UP|Q7XJ26 (Q7XJ26) Iron/ascorbate-dependent oxidoreductase	100	7.7e-37	TC190022	homologue to UP|Q7XJ26 (Q7XJ26) Iron/ascorbate-dependent oxidoreductase	3.2e-39
HV_CEb0006G24f	BE215306	UP|O82072 (O82072) Phospoenolpyruvate carboxylase	99.28	3.5e-58	TC206525	UP|O82072 (O82072) Phospoenolpyruvate carboxylase	1.9e-55
HV_CEb0006J08f	BE215358	UP|PR1A_HORVU (P32937) Pathogenesis-related protein 1A/1B precursor	100	1.0e-104	TC207050	homologue to UP|TLP_WHEAT (P27357) Thaumatin-like protein PWIR2 precursor	6.0e-96
HV_CEb0006L16f	BE215412	homologue to UP|Q93VK6 (Q93VK6) 3-phosphoshikimate 1-carboxyvinyltransferase (5-enolpyruvylshikimate 3-phosphate synthase)	95.06	8.2e-97	TC192224	homologue to UP|Q93VK6 (Q93VK6) 3-phosphoshikimate 1-carboxyvinyltransferase (5-enolpyruvylshikimate 3-phosphate synthase)	1.3e-93
HV_CEb0006L19f	BE215415	homologue to UP|Q84S63 (Q84S63) Putative serine/threonine kinase protein	90.58	1.6e-103	TC194646	similar to UP|Q84S63 (Q84S63) Putative serine/threonine kinase protein	5.7e-107
HV_CEb0006M15f	BE215434	UP|Q7XZU7 (Q7XZU7) GAD1	99.8	4.5e-80	TC220272	homologue to UP|P93369 (P93369) Glutamate decarboxylase	1.2e-130
HV_CEb0006M17f	BE215436	similar to UP|O82774 (O82774) Protein phosphatase 2A 55 kDa B regulatory subunit	89.72	1.2e-101	TC218836	homologue to UP|O82774 (O82774) Protein phosphatase 2A 55 kDa B regulatory subunit	2.4e-109
HV_CEb0006N09f	BE215450	similar to UP|Q6GYA9 (Q6GYA9) ABCF-type protein	89.43	3.3e-131	TC222618	homologue to UP|Q6GYA9 (Q6GYA9) ABCF-type protein	1.9e-30
HV_CEb0006P14f	unknown	no hit			no hit	no hit	
HV_CEb0007B02f	BE215595	weakly similar to UP|P93529 (P93529) Sorghum bicolor leucine-rich repeat-containing extracellular glycoprotein precursor	67.05	1.5e-113	TC226610	weakly similar to UP|P93529 (P93529) Sorghum bicolor leucine-rich repeat-containing extracellular glycoprotein precursor	2.8e-88
HV_CEb0007F10f[a]	BI955817	homologue to UP|Q9AXS1 (Q9AXS1) Putative thiamine biosythesis protein ThiC	96.7	2.6e-143	TC221721	homologue to UP|Q9AXS1 (Q9AXS1) Putative thiamine biosythesis protein ThiC	4.5e-124
HV_CEb0007H18f	BE215637	similar to UP|Q6ZII0 (Q6ZII0) Putative cytochrome P450	76.38	1.8e-105	TC191737	similar to UP|Q6ZIG8 (Q6ZIG8) Putative cytochrome P450	1.4e-56
HV_CEb0007L14f	BE215659	homologue to UP|P93852 (P93852) Endosperm C-24 sterol methyltransferase (EC 2.1.1.41)	93.9	4.0e-112	TC192488	homologue to UP|P93852 (P93852) Endosperm C-24 sterol methyltransferase (EC 2.1.1.41)	1.8e-89
HV_CEb0007N20f	BE215674	homologue to UP|Q84PB1 (Q84PB1) Putative phosphoribosylanthranilate transferase (Fragment)	92.68	6.2e-113	TC209483	homologue to UP|Q84PB1 (Q84PB1) Putative phosphoribosylanthranilate transferase (Fragment)	6.8e-105
HV_CEb0008A11f	BE215517	UP|KAO1_HORVU (Q9AXH9) Ent-kaurenoic acid oxidase 1 (gpr5) (EC 1.14.13.79)	100	4.1e-17	TC196068	homologue to UP|Q94IW5 (Q94IW5) Cytochrome P450-like protein	2.3e-95
HV_CEb0008B05f	BE215535	homologue to UP|Q75KK8 (Q75KK8) Putative Mitogen-activated protein kinase	98.15	3.4e-106	TC208063	homologue to UP|Q75KK8 (Q75KK8) Putative Mitogen-activated protein kinase	4.6e-94
HV_CEb0008B19f[a]	BE215535	homologue to UP|Q75KK8 (Q75KK8) Putative Mitogen-activated protein kinase	98.15	3.4e-106	TC208063	homologue to UP|Q75KK8 (Q75KK8) Putative Mitogen-activated protein kinase	4.6e-94
HV_CEb0008D10f	BE215587	homologue to UP|Q9SAU2 (Q9SAU2) Ribulose-5-phosphate-3-epimerase (EC 5.1.3.1)	93.13	1.8e-100	TC191020	homologue to UP|Q9SAU2 (Q9SAU2) Ribulose-5-phosphate-3-epimerase (EC 5.1.3.1)	1.2e-92
HV_CEb0008D24f	BE215697	homologue to SP|P43400 Metallothionein-like protein 1. [Wheat]	97.33	e-100	TC220016	UP|MT1_WHEAT (P43400) Metallothionein-like protein 1 (MT-1)	2.9e-73
HV_CEb0008F05f	unknown	no hit			no hit	no hit	
HV_CEb0008F10f	unknown	no hit			no hit	no hit	
HV_CEb0008H24f	BE215790	similar to GB|AAL16297 At1g79440/T8K14_14 {Arabidopsis thaliana;} (EC 1.2.1.24) succinate-semialdehyde dehydrogenase	78.53	3.7e-113	TC191784	similar to GB|AAL16297 At1g79440/T8K14_14 {Arabidopsis thaliana;} (EC 1.2.1.24) succinate-semialdehyde dehydrogenase	1.6e-109
HV_CEb0008L09f	BE215870	homologue to UP|Q8S1A8 (Q8S1A8) Putative casein kinase	91.76	1.3e-48	TC223337	homologue to UP|Q8S1A8 (Q8S1A8) Putative casein kinase	2.1e-47

Gene ID	Gene accession	Putative function Barley database	% Identity	Barley database hit	Wheat database blast	Putative function Wheat database	Wheat database hit
HV_CEb0008N11f	BE215917	homologue to UP|Q7XQP4 (Q7XQP4) OSJNBa0084A10.11 protein (Serine/threonine protein kinase SAPK7)	92.93	6.5e-110	TC187966	homologue to UP|Q7XQP4 (Q7XQP4) OSJNBa0084A10.11 protein (Serine/threonine protein kinase SAPK7)	1.4e-83
HV_CEb0008O07f	BE215937	homologue to GB|BAC07147 contains ESTs AU094020(E1880) AU094021(E1880) similar to protein kinase SRPK2	94.07	3.2e-104	TC218278	similar to UP|Q9FYB4 (Q9FYB4) SRPK1	1.2e-35
HV_CEb0009A03f	BE215980	homologue to UP|O04186 (O04186) Fd-GOGAT protein (Fragment)	93.65	1.3e-139	TC218941	homologue to UP|O04187 (O04187) Fd-GOGAT protein (Fragment)	1.3e-127
HV_CEb0009B05f	BE216003	homologue to UP|Q9FRT5 (Q9FRT5) Monosaccharide transporter 3	91.16	3.3e-116	TC207770	homologue to UP|Q9FRT5 (Q9FRT5) Monosaccharide transporter 3	1.1e-101
HV_CEb0009C15f	unknown	no hit			no hit	no hit	
HV_CEb0009C21f	BE216031	UP|Q9SWU5 (Q9SWU5) Receptor-like kinase	99.04	5.3e-140	NP411547	TAK14 [Triticum aestivum]	4.0e-87
HV_CEb0009D03f	BE216036	similar to UP|Q9XEN6 (Q9XEN6) Chitinase IV	85.55	1.0e-119	TC215989	UP|Q9XEN3 (Q9XEN3) Chitinase II	1.2e-93
HV_CEb0009E12f	AV931424	UP|THN7_HORVU (Q42838) Thionin BTH7 precursor	99.27	1.1e-136	TC220306	UP|Q9T0P1 (Q9T0P1) Alpha purothionin precursor	4.7e-47
HV_CEb0009E24f	BE216070	UP|FER_WHEAT (P00228) Ferredoxin chloroplast precursor	99.3	8.2e-31	TC190519	homologue to UP|Q75LK5 (Q75LK5) Putative ferredoxin	3.1e-92
HV_CEb0009H11f	AY037941	homologue to UP|Q75W16 (Q75W16) 3-deoxy-D-arabino heptulosonate-7-phosphate synthase	90	4.4e-54	TC220398	homologue to UP|Q75W16 (Q75W16) 3-deoxy-D-arabino heptulosonate-7-phosphate synthase	8.9e-26
HV_CEb0009I05f	AJ251298	homologue to UP|Q8H413 (Q8H413) Spermidine synthase 1	94.1	2.9e-147	TC220400	homologue to UP|Q8H413 (Q8H413) Spermidine synthase 1	1.4e-149
HV_CEb0009I06f	BE216122	similar to UP|Q9XEN6 (Q9XEN6) Chitinase IV	85.55	1.6e-120	TC225035	UP|Q9XEN6 (Q9XEN6) Chitinase IV	6.4e-81
HV_CEb0009I10f	BE216126	weakly similar to UP|P93529 (P93529) Sorghum bicolor leucine-rich repeat-containing extracellular glycoprotein precursor	67.05	2.1e-115	TC226610	weakly similar to UP|P93529 (P93529) Sorghum bicolor leucine-rich repeat-containing extracellular glycoprotein precursor	5.2e-95
HV_CEb0009K04f[a]	BE558918	homologue to UP|Q94IJ4 (Q94IJ4) Somatic embryogenesis receptor-like kinase 2 precursor	90.16	2.4e-29	TC202723	homologue to UP|Q84JK7 (Q84JK7) Leucine-rich repeat protein	4.6e-28
HV_CEb0009K15f	X95257	homologue to UP|XYLA_HORVU (Q40082) Xylose isomerase (EC 5.3.1.5)	98.54	0.0	TC220364	homologue to UP|XYLA_HORVU (Q40082) Xylose isomerase (EC 5.3.1.5 EC 5.3.1.5)	2.4e-191
HV_CEb0009K19f	BE216167	similar to GB|AAC49867 glyoxalase II cytoplasmic isozyme {Arabidopsis thaliana;}	71.13	1.4e-141	TC202871	similar to UP|GL2C_ARATH (O24496) Hydroxyacylglutathione hydrolase cytoplasmic (Glyoxalase II) (Glx II)	7.6e-129
HV_CEb0009O04f	BE216221	similar to UP|Q9J844 (Q9J844) ORF93 DNA polymerase	84.62	4.3e-127	TC228744	weakly similar to UP|O49571 (O49571) DNA topoisomerase like-protein	2.8e-119
HV_CEb0009O08f	BE216225	similar to UP|Q94IJ5 (Q94IJ5) SERK2 protein precursor	73.08	1.7e-120	TC226610	similar to UP|Q94IJ5 (Q94IJ5) SERK2 protein precursor	2.6e-98
HV_CEb0009O11f	BE216228	homologue to UP|Q8LP96 (Q8LP96) Moco containing protein (Moco containing protein(OsMCP))	91.94	1.8e-143	TC193475	homologue to UP|Q8LP96 (Q8LP96) Moco containing protein (Moco containing protein(OsMCP))	1.3e-136
HV_CEb0010B21f	BE216294	homologue to UP|Q7F280 (Q7F280) NADP-specific isocitrate dehydrogenase (EC 1.1.1.42)	94.13	1.8e-174	TC188690	homologue to UP|Q7F280 (Q7F280) NADP-specific isocitrate dehydrogenase (EC 1.1.1.42)	1.8e-160
HV_CEb0010C04f	BE216301	weakly similar to PIR|G84912 probable acyl-CoA synthetase [imported] - Arabidopsis thaliana {Arabidopsis thaliana;} (EC 6.2.1.3)	69.74	2.7e-156	TC225561	weakly similar to PIR|G84912 probable acyl-CoA synthetase [imported] - Arabidopsis thaliana {Arabidopsis thaliana;} (EC 6.2.1.3)	3.7e-136
HV_CEb0010C13f	BE216310	similar to UP|Q43700 (Q43700) Heat shock factor	84.29	3.1e-118	TC201353	similar to UP|Q6K6S5 (Q6K6S5) Putative heat stress transcription factor Spl7	4.5e-33
HV_CEb0010E08f	BE216352	similar to UP|Q7XHB3 (Q7XHB3) Putative peroxidase	73.81	1.7e-151	|TC204694	similar to UP|Q7XHB3 (Q7XHB3) Putative peroxidase	3.9e-103
HV_CEb0010F20f	BE216388	homologue to UP|Q8LNZ3 (Q8LNZ3) UDP-glucose 4-epimerase	90.54	6.6e-128	TC206569	homologue to UP|Q8LNZ3 (Q8LNZ3) UDP-glucose 4-epimerase	2.7e-105
HV_CEb0010F21f[a]	BE216388	homologue to UP|Q8LNZ3 (Q8LNZ3) UDP-glucose 4-epimerase	90.54	6.6e-128	TC206569	homologue to UP|Q8LNZ3 (Q8LNZ3) UDP-glucose 4-epimerase	2.7e-105
HV_CEb0010G02f	BE216394	UP|Q711Q3 (Q711Q3) Cathepsin B	100	8.3e-146	TC220218	UP|Q03106 (Q03106) Cathepsin B (Fragment) (EC 3.4.22.-)	1.3e-138
HV_CEb0010G09f	BE216401	similar to GB|BAC22396 putative indole-3-glycerol phosphate synthase {Oryza sativa (japonica cultivar-group);}	87.12	3.3e-147	TC208882	similar to GB|BAC22396 putative indole-3-glycerol phosphate synthase {Oryza sativa (japonica cultivar-group);}	4.4e-136
HV_CEb0010G19f	BE216411	UP|E13B_HORVU (P15737) Glucan endo-1 3-beta-glucosidase GII precursor	100	9.7e-55	BI480524	UP|Q9XEN5 (Q9XEN5) Beta-1 3-glucanase	e-112

Gene ID	Gene accession	Putative function Barley database	% Identity	Barley database hit	Wheat database blast	Putative function Wheat database	Wheat database hit
HV_CEb0010H13f	BE216428	similar to UP|Q8H841 (Q8H841) Putative receptor-like protein kinase	75.61	2.3e-154	BE443808	similar to UP|Q8H841 (Q8H841) Putative receptor-like protein kinase	e-141
HV_CEb0010J22f	BE216485	homologue to UP|Q7GC12 (Q7GC12) Shaggy-related protein kinase gamma	95.32	4.8e-150	TC218796	homologue to UP|Q7GC12 (Q7GC12) Shaggy-related protein kinase gamma	3.3e-143
HV_CEb0010L20f	BE216529	UP|PR1_HORVU (Q05968) Pathogenesis-related protein 1 precursor	100	0.0	BF474571	UP|Q94F73 (Q94F73) Pathogenesis-related protein 1	0.0
HV_CEb0010M14f[a]	BE216529	UP|PR1_HORVU (Q05968) Pathogenesis-related protein 1 precursor	100	3.5e-147	BF474571	UP|Q94F73 (Q94F73) Pathogenesis-related protein 1	7.3e-105
HV_CEb0010N01f	BE216558	homologue to UP|FDH_HORVU (Q9ZRI8) Formate dehydrogenase mitochondrial precursor (NAD-dependent formate dehydrogenase) (FDH)	98.41	3.8e-160	TC189543	homologue to UP|FDH_HORVU (Q9ZRI8) Formate dehydrogenase mitochondrial precursor (NAD-dependent formate dehydrogenase) (FDH)	2.1e-152
HV_CEb0010N04f	BE216561	homologue to UP|Q41328 (Q41328) Pto kinase interactor 1	93.91	2.0e-25	CK215577	weakly similar to GP|29838544 protein kinase Pti1 {Glycine max}	1.5e-109
HV_CEb0010N06f	BE216563	homologue to GP|17981573 kinase R-like protein {Triticum aestivum}	92.42	1.8e-20	AF445791.1	kinase R-like protein [Triticum aestivum]	7.6e-46
HV_CEb0010N22f	BE216579	similar to UP|Q6YUU3 (Q6YUU3) Putative leucine-rich repeat transmembrane protein kinase	87.38	5.4e-147	TC208946	similar to UP|Q6YUU3 (Q6YUU3) Putative leucine-rich repeat transmembrane protein kinase	2.9e-104
HV_CEb0010O15f	BE216596	similar to PIR|A86347 branched-chain alpha keto-acid dehydrogenase E1-alpha subunit [imported] - Arabidopsis thaliana	73.92	1.3e-151	TC218468	similar to PIR|A86347 branched-chain alpha keto-acid dehydrogenase E1-alpha subunit [imported] - Arabidopsis thaliana	2.3e-142
HV_CEb0010O15f[a]	BE216802	homologue to UP|Q94DH6 (Q94DH6) Putative cytochrome B5	90.7	2.2e-28	TC192439	homologue to UP|Q94DH6 (Q94DH6) Putative cytochrome B5	1.1e-29
HV_CEb0010P06f	BE216610	homologue to UP|Q43220 (Q43220) Peroxidase (EC 1.11.1.7)	93.82	5.7e-37	TC193381	UP|Q43220 (Q43220) Peroxidase (EC 1.11.1.7)	2.5e-75
HV_CEb0010P06f[a]	BE216802	homologue to UP|Q94DH6 (Q94DH6) Putative cytochrome B6	90.71	2.2e-28	TC192439	homologue to UP|Q94DH6 (Q94DH6) Putative cytochrome B6	1.1e-29
HV_CEb0011B05f	BE216643	similar to UP|Q6ZD35 (Q6ZD35) Putative glycyl-tRNA synthetase	80.38	2.8e-123	TC221027	similar to UP|Q6ZD35 (Q6ZD35) Putative glycyl-tRNA synthetase	1.3e-110
HV_CEb0011B08f	BE216646	similar to GP|19849279 Cyt-P450 monooxygenase [Oryza sativa (japonica cultivar-group)]	80.67	3.5e-219	TC194104	similar to UP|Q6Z3F0 (Q6Z3F0) Cyt-P450 monooxygenase	2.1e-99
HV_CEb0011F02f[a]	BE216529	UP|PR1_HORVU (Q05968) Pathogenesis-related protein 1 precursor	100	3.5e-147	BF474571	UP|Q94F73 (Q94F73) Pathogenesis-related protein 1	7.3e-105
HV_CEb0011F16f	BE216724	homologue to UP|Q8W4U9 (Q8W4U9) Clathrin assembly protein AP17-like protein	97.87	1.2e-91	TC221346	homologue to UP|Q8W4U9 (Q8W4U9) Clathrin assembly protein AP17-like protein	3.6e-76
HV_CEb0011G08f	BE216736	similar to UP|O23637 (O23637) Argininosuccinate lyase (EC 4.3.2.1)	77.57	3.0e-145	TC208754	similar to UP|O23637 (O23637) Argininosuccinate lyase (EC 4.3.2.1)	6.5e-135
HV_CEb0011I04f	BE216768	similar to UP|Q84S61 (Q84S61) Putative serine/threonine kinase protein	81.77	1.5e-66	TC212991	similar to UP|Q84S61 (Q84S61) Putative serine/threonine kinase protein	2.6e-81
HV_CEb0011K14f	BE216808	weakly similar to UP|P93529 (P93529) Sorghum bicolor leucine-rich repeat-containing extracellular glycoprotein precursor	67.05	4.9e-151	TC226610	weakly similar to UP|P93529 (P93529) Sorghum bicolor leucine-rich repeat-containing extracellular glycoprotein precursor	3.5e-118
HV_CEb0011N15f	BE216860	P|Q43482 (Q43482) High affinity sulphate transporter	100	8.3e-21	TC223956	UP|Q8H0K3 (Q8H0K3) Sulphate transporter	7.6e-20
HV_CEb0015B10f	BE558194	weakly similar to GP|22535653 putative protein kinase Xa21 receptor type precursor {Oryza sativa (japonica cultivar-group)}	68.94	1.6e-113	AY072046.1	Xa-21 resistance-receptor kinase-like protein [Triticum aestivum]	5.2e-16
HV_CEb0015D24f	BE558211	homologue to UP|Q75GF5 (Q75GF5) Putative general negative regulator of transcription subunit 3'-partial (Fragment)	97.55	7.0e-115	BQ804162	homologue to GP|9758905 contains similarity to transcription regulator~gene_id:MRG7.19 {Arabidopsis thaliana}	2.7e-47
HV_CEb0016D06f	BE519575	similar to GP|16604649 putative protein kinase {Arabidopsis thaliana}	81.72	8.3e-112	TC200267	similar to UP|Q9C902 (Q9C902) Protein kinase putative; 19229-23534 (Hypothetical protein At3g06620)	3.1e-35
HV_CEb0016L13f	BE519515	homologue to UP|Q9LLD7 (Q9LLD7) Fructose 1 6-bisphosphate aldolase (EC 4.1.2.13)	96.9	2.5e-149	TC218353	homologue to UP|Q9LLD7 (Q9LLD7) Fructose 1 6-bisphosphate aldolase (EC 4.1.2.13)	1.8e-140
HV_CEb0016L17f	BE519519	similar to UP|Q7F169 (Q7F169) S-receptor kinase PK3-like protein	86.11	2.0e-106	BE414756	weakly similar to GP|10177548 receptor protein kinase-like protein {Arabidopsis thaliana}	5.0e-15
HV_CEb0016N18f	BE519542	UP|Q9SME4 (Q9SME4) Glutathione peroxidase-like protein GPX54Hv	100	7.1e-101	TC202747	UP|Q9SME4 (Q9SME4) Glutathione peroxidase-like protein GPX54Hv	1.3e-83
HV_CEb0017A03f	TAU48692	UP|P94058 (P94058) Calmodulin TaCaM2-3 (Calmodulin TaCaM2-2)	100	5.7e-158	TC206817	UP|P94058 (P94058) Calmodulin TaCaM2-3 (Calmodulin TaCaM2-2)	7.5e-185
HV_CEb0017B21f	BE558296	similar to UP|Q7XH17 (Q7XH17) Putative receptor-like protein kinase 4	80.88	0.0	BE442802	similar to UP|Q7XH17 (Q7XH17) Putative receptor-like protein kinase 4	0

Gene ID	Gene accession	Putative function Barley database	% Identity	Barley database hit	Wheat database blast	Putative function Wheat database	Wheat database hit
HV_CEb0017G11f[a]	BE216802	homologue to UP|Q94DH6 (Q94DH6) Putative cytochrome B5	90.7	2.2e-28	TC192439	homologue to UP|Q94DH6 (Q94DH6) Putative cytochrome B5	1.1e-29
HV_CEb0017H19f	BE558391	similar to UP|Q94IP1 (Q94IP1) Cinnamic acid 4-hydroxylase (EC 1.14.13.11)	89.62	1.7e-100	TC190513	UP|Q9FVM9 (Q9FVM9) Cytochrome P450 homologue to UP|Q94IP1 (Q94IP1) Cinnamic acid 4-hydroxylase (EC 1.14.13.11)	1.2e-99
HV_CEb0017J10f[a]	BI952869	homologue to UP|Q8LNZ3 (Q8LNZ3) UDP-glucose 4-epimerase	90.54	3.3e-62	TC206570	homologue to UP|Q8LNZ3 (Q8LNZ3) UDP-glucose 4-epimerase	1.4e-58
HV_CEb0017L05f	BE558442	weakly similar to UP|Q84NG8 (Q84NG8) Putative receptor kinase	63.39	2.0e-40	CA696411	weakly similar to GP|22535653 putative protein kinase Xa21 receptor type precursor {Oryza sativa (japonica cultivar-group)}	7.7e-37
HV_CEb0018E04f	BE519673	similar to UP|Q8RUD7 (Q8RUD7) Similar to protein kinase AtSIK (P0485B12.21 protein)	86.95	8.8e-110	TC217635	similar to UP|Q84SA6 (Q84SA6) Serine/threonine protein kinase	0.00018
HV_CEb0018F13f	BE558536	similar to UP|SOD4_MAIZE (P23345) Superoxide dismutase [Cu-Zn] 4A (EC 1.15.1.1)	89.4	4.5e-154	TC218333	homologue to UP|SOD4_MAIZE (P23345) Superoxide dismutase [Cu-Zn] 4A (EC 1.15.1.1 EC 1.15.1.1)	9.5e-123
HV_CEb0018H13f	BE558546	UP|CAHC_HORVU (P40880) Carbonic anhydrase chloroplast precursor (Carbonate dehydratase)	100	6.8e-154	TC218376	homologue to UP|CAHC_HORVU (P40880) Carbonic anhydrase chloroplast precursor (Carbonate dehydratase)	1.2e-143
HV_CEb0018K12f	AV836098	homologue to UP|Q9LD61 (Q9LD61) Aspartate carbamoyl transferase	95.48	8.4e-142	TC203283	homologue to UP|Q9LD61 (Q9LD61) Aspartate carbamoyl transferase	2.8e-123
HV_CEb0018M20f	BE519721	similar to UP|O23254 (O23254) Serine hydroxymethyltransferase	84.58	6.5e-111	TC218818	similar to UP|O23254 (O23254) Serine hydroxymethyltransferase	1.7e-106
HV_CEb0020A17f[a]	BE558697	homologue to UP|KPPR_WHEAT (P26302) Phosphoribulokinase chloroplast precursor (Phosphopentokinase) (PRKASE) (PRK)	98.35	2.4e-31	TC206138	UP|KPPR_WHEAT (P26302) Phosphoribulokinase chloroplast precursor (Phosphopentokinase) (PRKASE) (PRK)	7.7e-29
HV_CEb0020C05f	BE558708	UP|Q84UD4 (Q84UD4) GAMYB-binding protein	99.79	1.9e-29	TC191498	homologue to UP|Q84UD4 (Q84UD4) GAMYB-binding protein	3.0e-29
HV_CEb0020C14f[a]	BE558708	UP|Q84UD4 (Q84UD4) GAMYB-binding protein	99.79	1.9e-29	TC191498	homologue to UP|Q84UD4 (Q84UD4) GAMYB-binding protein	3.0e-29
HV_CEb0020E03f	BE558748	homologue to UP|UBA2_WHEAT (P31251) Ubiquitin-activating enzyme E1 2	98.83	2.7e-87	TC206889	UP|UBA2_WHEAT (P31251) Ubiquitin-activating enzyme E1 2	1.1e-64
HV_CEb0020E08f	BE558753	similar to UP|Q6K2E1 (Q6K2E1) Putative UDP-glucose 4-epimerase	88.21	5.6e-167	TC206570	homologue to UP|Q8LNZ3 (Q8LNZ3) UDP-glucose 4-epimerase	4.5e-58
HV_CEb0020G04f[a]	BE216074	homologue to GP|15289978 putative cytochrome B5 {Oryza sativa (japonica cultivar-group)}	93.08	6.2e-117	TC189309	homologue to UP|Q94DH6 (Q94DH6) Putative cytochrome B5	1.1e-91
HV_CEb0020G11f	BE558794	similar to GP|11762214 AT3g04520 {Arabidopsis thaliana} L-allo-threonine aldolase like protein	72.46	8.8e-140	TC220779	similar to UP|GLY1_SCHPO (O13940) Probable threonine aldolase (EC 4.1.2.5)	1.2e-112
HV_CEb0020H16f	BE558822	homologue to UP|FDH_HORVU (Q9ZRI8) Formate dehydrogenase mitochondrial precursor (NAD-dependent formate dehydrogenase)	98.41	4.9e-92	CK207030	homologue to SP|Q9ZRI8 Formate dehydrogenase mitochondrial precursor (EC 1.2.1.2) (NAD- dependent formate dehydrogenase)	5.0e-80
HV_CEb0020J06f	BE558852	homologue to UP|Q7Y1F0 (Q7Y1F0) Putative glycine hydroxymethyltransferase	93.74	1.8e-136	TC218524	homologue to UP|Q7Y1F0 (Q7Y1F0) Putative glycine hydroxymethyltransferase	3.4e-144
HV_CEb0020K09f	BE558877	homologue to UP|Q94F82 (Q94F82) Histone deacetylase HDA101	91.43	9.1e-169	TC188791	homologue to UP|Q94F82 (Q94F82) Histone deacetylase HDA101	7.6e-162
HV_CEb0020M10f	BE558918	homologue to UP|Q94IJ4 (Q94IJ4) Somatic embryogenesis receptor-like kinase 2 precursor	90.16	2.4e-29	TC202723	homologue to UP|Q84JK7 (Q84JK7) Leucine-rich repeat protein	4.6e-28
HV_CEb0020M17f	BE558923	similar to UP|Q9LQN8 (Q9LQN8) F24B9.29 protein	80	1.6e-159	TC212957	similar to UP|Q9LQN8 (Q9LQN8) F24B9.29 protein	5.3e-51
HV_CEb0020M23f	BE558929	homologue to UP|Q7FSL4 (Q7FSL4) Malate dehydrogenase	90.85	9.5e-123	TC220055	similar to UP|Q7FSL4 (Q7FSL4) Malate dehydrogenase	7.6e-114
HV_CEb0021A10f[a]	AC073556	similar to UP|TCMO_ZINEL (Q43240) Trans-cinnamate 4-monooxygenase	83.93	3.7e-176	TC190513	homologue to UP|Q94IP1 (Q94IP1) Cinnamic acid 4-hydroxylase (EC 1.14.13.11)	1.2e-99
HV_CEb0021A21f[a]	AB070252	homologue to UP|PRS7_ORYSA (Q9FXT9) 26S protease regulatory subunit 7	97.42	2.0e-114	TC202839	homologue to UP|PRS7_ORYSA (Q9FXT9) 26S protease regulatory subunit 7 (26S proteasome subunit 7)	2.5e-113
HV_CEb0021B09f	BE519751	similar to UP|Q9ATU7 (Q9ATU7) Putative cytochrome P450	71.15	4.5e-149	TC215821	similar to UP|Q9ATU7 (Q9ATU7) Putative cytochrome P450	5.3e-68
HV_CEb0021C11f	BE519766	UP|Q70EZ8 (Q70EZ8) Putative 1-deoxy-D-xylulose 5-phosphate reductoisomerase precursor (EC 1.1.1.267)	100	5.8e-126	TC221843	homologue to UP|Q70EZ8 (Q70EZ8) Putative 1-deoxy-D-xylulose 5-phosphate reductoisomerase precursor (EC 1.1.1.267)	8.2e-119

Gene ID	Gene accession	Putative function Barley database	% Identity	Barley database hit	Wheat database blast	Putative function Wheat database	Wheat database hit
HV_CEb0021F09f	BE519816	similar to UP|Q8S091 (Q8S091) Putative thioredoxin	85.71	3.3e-147	TC221862	similar to UP|Q8S091 (Q8S091) Putative thioredoxin	8.3e-132
HV_CEb0021G05f	BE216563	homologue to GP|17981573 kinase R-like protein {Triticum aestivum}	92.42	1.8e-20	AF445791.1	kinase R-like protein [Triticum aestivum]	7.6e-46
HV_CEb0021J19f	BE519892	similar to UP|Q8H8H7 (Q8H8H7) Putative flavanone 3-hydroxylase	89.19	1.3e-138	TC203115	similar to UP|Q9FLV0 (Q9FLV0) Flavanone 3-hydroxylase-like protein	2.4e-73
HV_CEb0021K16f	BE519908	homologue to UP|Q6YT73 (Q6YT73) Putative glycolate oxidase	94.55	5.5e-147	TC190248	similar to UP|Q6YT73 (Q6YT73) Putative glycolate oxidase	2.5e-128
HV_CEb0021M24f	BE215595	weakly similar to UP|P93529 (P93529) Sorghum bicolor leucine-rich repeat-containing extracellular glycoprotein precursor	67.05	1.5e-113	TC226610	weakly similar to UP|P93529 (P93529) Sorghum bicolor leucine-rich repeat-containing extracellular glycoprotein precursor	2.8e-88
HV_CEb0021O16f	BE519974	similar to UP|Q7XHB3 (Q7XHB3) Putative peroxidase	73.81	9.1e-150	TC204694	similar to UP|Q7XHB3 (Q7XHB3) Putative peroxidase	4.2e-109
HV_CEb0021P01f	BE519980	UP|Q40068 (Q40068) Peroxidase (EC 1.11.1.7)	100	6.9e-152	TC224463	UP|Q43212 (Q43212) Peroxidase precursor	1.5e-107
HV_CEb0022D14f	AB033535	homologue to UP|PRS7_ORYSA (Q9FXT9) 26S protease regulatory subunit 7	98.36	4.8e-221	TC202839	homologue to UP|PRS7_ORYSA (Q9FXT9) 26S protease regulatory subunit 7 (26S proteasome subunit 7)	3.0e-196
HV_CEb0022L14f[a]	BE216724	homologue to UP|Q8W4U9 (Q8W4U9) Clathrin assembly protein AP17-like protein	97.87	1.2e-91	TC221346	homologue to UP|Q8W4U9 (Q8W4U9) Clathrin assembly protein AP17-like protein	3.6e-76
HV_CEb0022N20f	BE559104	UP|O22575 (O22575) Glycine decarboxylase P subunit (EC 1.4.4.2)	99.37	1.4e-151	TC220061	UP|O22575 (O22575) Glycine decarboxylase P subunit (EC 1.4.4.2)	3.3e-147
HV_CEb0022O04f	BE559108	homologue to UP|Q94DH6 (Q94DH6) Putative cytochrome B5	93.08	2.5e-137	TC188689	homologue to UP|Q94DH6 (Q94DH6) Putative cytochrome B5	2.4e-99
HV_CEb0023C21f[a]	BG418805	homologue to UP|FKB7_WHEAT (Q43207) 70 kDa peptidylprolyl isomerase (Peptidyl-prolyl cis-trans isomerase) (PPIase) (Rotamase)	97.32	2.2e-106	TC220854	homologue to UP|FKB7_WHEAT (Q43207) 70 kDa peptidylprolyl isomerase (Peptidyl-prolyl cis-trans isomerase) (PPIase) (Rotamase)	1.7e-88
HV_CEb0023F16f	BE559272	homologue to UP|STAD_ORYSA (Q40731) Acyl-[acyl-carrier-protein] desaturase chloroplast precursor (Stearoyl-ACP desaturase)	92.1	6.0e-171	TC191406	homologue to UP|STAD_ORYSA (Q40731) Acyl-[acyl-carrier-protein] desaturase chloroplast precursor (Stearoyl-ACP desaturase)	6.5e-165
HV_CEb0023H18f	BE559296	similar to UP|Q42810 (Q42810) GmCK2p (EC 2.7.1.32) choline kinase	70.64	8.6e-155	TC211069	similar to UP|Q8S1L7 (Q8S1L7) Putative choline kinase	5.7e-111
HV_CEb0024D15f	BE559347	similar to UP|Q9FW09 (Q9FW09) Putative ferulate-5-hydroxylase	87.01	1.7e-112	TC210375	similar to UP|Q9FW09 (Q9FW09) Putative ferulate-5-hydroxylase	5.3e-39
HV_CEb0024H02f	BE559387	UP|Q43764 (Q43764) Chitinase (EC 3.2.1.14)	100	7.5e-137	TC209470	UP|Q8W429 (Q8W429) Chitinase 1.complete	2.5e-137
HV_CEb0024H14f	BE559397	UP|PR12_HORVU (P35792) Pathogenesis-related protein PRB1-2 precursor	99.39	0.0	BF474571	UP|Q94F73 (Q94F73) Pathogenesis-related protein 1	0.0
HV_CEb0024L17f	BE559447	homologue to UP|Q6K306 (Q6K306) Putative indole-3-glycerol phosphate synthase	90.51	9.2e-108	TC197077	homologue to UP|Q6K306 (Q6K306) Putative indole-3-glycerol phosphate synthase	9.6e-76
HV_CEb0024M02f	BE559451	similar to UP|Q8H7X7 (Q8H7X7) Putative sulfate transporter ATST1	86.84	4.3e-112	TC209273	similar to UP|Q8H7X7 (Q8H7X7) Putative sulfate transporter ATST1	1.7e-101
HV_CEb0024N06f	BE559467	homologue to UP|Q8GU89 (Q8GU89) PDR-like ABC transporter (PDR4 ABC transporter)	91.23	1.1e-102	TC209797	homologue to UP|Q8GU89 (Q8GU89) PDR-like ABC transporter (PDR4 ABC transporter)	1.7e-64
HVSMEg0001A02f	BE230858	homologue to UP|Q84N28 (Q84N28) Caffeic acid O-methyltransferase	98.33	2.7e-115	TC191033	UP|Q84N28 (Q84N28) Caffeic acid O-methyltransferase	2.7e-101
HVSMEg0001G18f	BE230890	UP|Q94L27 (Q94L27) Alcohol dehydrogenase	99.47	9.9e-126	TC188649	homologue to UP|Q94L27 (Q94L27) Alcohol dehydrogenase	1.9e-115
HVSMEg0001J18f	BG342993	UP|Q9FPR4 (Q9FPR4) EDR1 Enhanced disease resistance 1	99.9	1.2e-24	TC209240	homologue to UP|Q6YW44 (Q6YW44) Putative MAP3K delta-1 protein kinase	2.4e-138
HVSMEg0001K11f	BG343000	similar to PIR|T05536 acid phosphatase (EC 3.1.3.2) - Arabidopsis thaliana	81.82	2.8e-80	TC219352	homologue to UP|Q6Z3C0 (Q6Z3C0) Acid phosphatase-like	6.2e-10
HVSMEg0001N16f	BG343051	similar to UP|Q6Q1B9 (Q6Q1B9) CCAAT-box transcription factor complex WHAP12	82.35	7.1e-167	TC196948	similar to UP|Q6Q1B8 (Q6Q1B8) CCAAT-box transcription factor complex WHAP13	2.0e-131
HVSMEg0001P11f	BF261118	similar to UP|Q9FYP0 (Q9FYP0) Putative peroxidase	71.96	9.7e-155	TC192994	weakly similar to UP|Q9FYP0 (Q9FYP0) Putative peroxidase	6.7e-125
HVSMEg0002E08f	AW982203	homologue to UP|Q8SA22 (Q8SA22) Putative pyruvate kinase	95.83	1.5e-101	TC209247	homologue to UP|Q8SA22 (Q8SA22) Putative pyruvate kinase	8.4e-91
HVSMEg0002E11f	AW982206	similar to UP|Q6Z7L1 (Q6Z7L1) Putative dnaK-type molecular chaperone	83.76	6.3e-175	TC190247	similar to UP|Q6Z7L1 (Q6Z7L1) Putative dnaK-type molecular chaperone	1.2e-165
HVSMEg0002G09f	AW982228	homologue to UP|Q75RZ2 (Q75RZ2) Putative caffeoyl CoA O-methyltransferase	94.4	1.2e-163	TC206309	homologue to UP|Q75RZ2 (Q75RZ2) Putative caffeoyl CoA O-methyltransferase	2.0e-138
HVSMEg0002G13f	AW982232	UP|Q9MAY8 (Q9MAY8) Endo-1 4-beta-glucanase Cel1	100	2.3e-162	TC202736	UP|Q9MAY8 (Q9MAY8) Endo-1 4-beta-glucanase Cel1	2.8e-148

Gene ID	Gene accession	Putative function Barley database	% Identity	Barley database hit	Wheat database blast	Putative function Wheat database	Wheat database hit
HVSMEg0002G20f	AW982239	similar to UP|Q9AVE7 (Q9AVE7) Zeaxanthin epoxidase	89.4	2.0e-158	TC222807	similar to UP|Q8W3L2 (Q8W3L2) Zeaxanthin epoxidase	3.5e-146
HVSMEg0002I02f	AW982245	UP|CAT2_HORVU (P55308) Catalase isozyme 2 (EC 1.11.1.6)	99.6	1.0e-86	TC188750	UP|CAT2_HORVU (P55308) Catalase isozyme 2 (EC 1.11.1.6 EC 1.11.1.6 EC 1.11.1.6)	1.6e-48
HVSMEg0002K13f	AW982280	similar to UP|Q8S7S6 (Q8S7S6) Cytochrome P450-like protein	74.46	5.2e-127	TC200083	similar to UP|Q8S7S6 (Q8S7S6) Cytochrome P450-like protein	1.3e-53
HVSMEg0002L08f	BG343142	similar to UP|Q944G0 (Q944G0) Mevalonate disphosphate decarboxylase	77.56	9.4e-169	TC208555	similar to UP|O23722 (O23722) Mevalonate diphosphate decarboxylase (At2g38700) (EC 4.1.1.33 EC 4.1.1.33)	6.0e-155
HVSMEg0002M03f	AW982294	homologue to UP|Q6H4P7 (Q6H4P7) Putative leucyl-tRNA synthetase	90.91	1.9e-164	TC222314	similar to UP|Q6H4P7 (Q6H4P7) Putative leucyl-tRNA synthetase	1.3e-139
HVSMEg0002M11f	AW982302	similar to UP|Q9FVD3 (Q9FVD3) Hexokinase	75	1.3e-124	TC223392	similar to UP|Q9FVD3 (Q9FVD3) Hexokinase	4.1e-106
HVSMEg0002M15f	AW982306	homologue to UP|Q6RS97 (Q6RS97) LON1 protease	98.42	6.0e-137	TC207614	UP|Q6RS97 (Q6RS97) LON1 protease	7.5e-127
HVSMEg0002N04f	BG343151	UP|Q40001 (Q40001) Protoporphyrin IX Mg-chelatase subunit precursor (EC 4.99.1.-)	99.78	2.4e-168	TC222136	UP|Q94C01 (Q94C01) Mg-chelatase subunit XANTHA-F	6.1e-88
HVSMEg0002N10f	BG343154	homologue to UP|O22664 (O22664) Cytosolic heat shock 70 protein	92.95	5.3e-162	TC220486	UP|Q9SAU8 (Q9SAU8) HSP70	2.5e-123
HVSMEg0002N16f	BG343157	homologue to UP|Q6K4K9 (Q6K4K9) Phosphatase 2A regulatory A subunit	95.91	3.0e-165	TC206215	homologue to UP|Q6K4K9 (Q6K4K9) Phosphatase 2A regulatory A subunit	1.0e-153
HVSMEg0002O09f	AW982323	homologue to UP|Q9LKM0 (Q9LKM0) Nucleoside diphosphate kinase (EC 2.7.4.6)	96.67	4.3e-140	TC218410	homologue to UP|Q9LKM0 (Q9LKM0) Nucleoside diphosphate kinase (EC 2.7.4.6)	3.0e-129
HVSMEg0002O22f[a]	BE231062	homologue to UP|Q9LKM0 (Q9LKM0) Nucleoside diphosphate kinase (EC 2.7.4.6)	96.67	3.7e-150	TC218410	homologue to UP|Q9LKM0 (Q9LKM0) Nucleoside diphosphate kinase (EC 2.7.4.6)	2.1e-134
HVSMEg0003C20f	AW982402	homologue to UP|Q8RYB1 (Q8RYB1) Porphobilinogen deaminase (Fragment)	95.78	5.2e-176	TC207371	UP|Q8RYB1 (Q8RYB1) Porphobilinogen deaminase (Fragment)	1.1e-141
HVSMEg0003D01f	AW982407	similar to UP|Q84PX0 (Q84PX0) Putative tyrosyl-tRNA synthetase	84.18	1.2e-133	TC202957	similar to UP|Q84PX0 (Q84PX0) Putative tyrosyl-tRNA synthetase	8.4e-129
HVSMEg0003D05f	AW982411	weakly similar to UP|Q75IK0 (Q75IK0) Putative o-methyltransferase ZRP4	69	3.7e-135	TC196208	similar to UP|ZRP4_MAIZE (P47917) O-methyltransferase ZRP4 (OMT) (EC 2.1.1.- EC 2.1.1.- EC 2.1.1.-)	5.5e-58
HVSMEg0003G20f	AW982497	UP|Q7XJ80 (Q7XJ80) Cytosolic heat shock protein 90	100	5.6e-174	TC190352	UP|Q7XJ80 (Q7XJ80) Cytosolic heat shock protein 90	2.9e-170
HVSMEg0003G22f	AW982499	homologue to UP|Q94GF1 (Q94GF1) Anthranilate synthase alpha 1 subunit	92.22	9.7e-129	TC223227	similar to UP|Q94GF1 (Q94GF1) Anthranilate synthase alpha 1 subunit	2.3e-113
HVSMEg0003I12f	AW982536	homologue to UP|Q6RK07 (Q6RK07) UDP-glucose dehydrogenase	91.04	3.7e-109	TC204306	homologue to UP|Q6RK07 (Q6RK07) UDP-glucose dehydrogenase	7.1e-101
HVSMEg0003I16f	AW982539	homologue to UP|Q84VF7 (Q84VF7) Putative receptor protein kinase (Fragment)	91.25	1.1e-74	TC192060	similar to UP|Q8W0B8 (Q8W0B8) Putative receptor protein kinase PERK1	2.6e-141
HVSMEg0003J10f	AW982555	homologue to UP|O49913 (O49913) Aquaporin	93.99	5.8e-54	TC188087	similar to UP|Q70AP3 (Q70AP3) Aquaporin	8.4e-102
HVSMEg0003M07f	AW982621	UP|Q6RYF4 (Q6RYF4) Coatomer alpha subunit	100	2.7e-142	TC219934	homologue to UP|Q6RYF4 (Q6RYF4) Coatomer alpha subunit	4.8e-125
HVSMEg0003N01f	AW982639	similar to UP|Q84PC6 (Q84PC6) Calmodulin-related protein (Fragment)	71.43	3.1e-142	TC203709	similar to UP|Q84PC6 (Q84PC6) Calmodulin-related protein (Fragment)	3.4e-113
HVSMEg0003N10f	BI951269	similar to GP|21553536 receptor-like protein kinase {Arabidopsis thaliana}	73.33	2.0e-159	TC212550	similar to UP|Q9AWX9 (Q9AWX9) Putative receptor-like protein	1.8e-63
HVSMEg0003N15f	AW982653	UP|Q8LK43 (Q8LK43) GSK-like kinase	99.73	1.1e-73	TC192583	UP|Q8LK43 (Q8LK43) GSK-like kinase	1.8e-74
HVSMEg0003O15f	AW982677	UP|CHS1_HORVU (P26018) Chalcone synthase 1 (Naringenin-chalcone synthase 1)	100	2.3e-100	TC206537	UP|CHS2_SECCE (P53415) Chalcone synthase 2 (Naringenin-chalcone synthase 2) (EC 2.3.1.74 EC 2.3.1.74)	1.5e-110
HVSMEg0004A22f	AW982731	homologue to UP|Q94C43 (Q94C43) Thiosulfate transferase	97.39	2.1e-118	TC193609	UP|Q94C43 (Q94C43) Thiosulfate transferase	2.6e-110
HVSMEg0004C01f	AW982753	UP|Q9SME4 (Q9SME4) Glutathione peroxidase-like protein GPX54Hv	100	2.7e-120	TC202747	UP|Q6UQ05 (Q6UQ05) Cytosolic glutathione peroxidase	2.3e-109
HVSMEg0004D14f	AW982790	UP|Q8LK43 (Q8LK43) GSK-like kinase (Glycogen synthase kinase-like kinase)	99.73	1.5e-88	TC192583	UP|Q8LK43 (Q8LK43) GSK-like kinase (Glycogen synthase kinase-like kinase)	1.1e-90

Gene ID	Gene accession	Putative function Barley database	% Identity	Barley database hit	Wheat database blast	Putative function Wheat database	Wheat database hit
HVSMEg0004E10f	AW982810	homologue to UP|LEU3_SOLTU (P29696) 3-isopropylmalate dehydrogenase chloroplast precursor (Beta-IPM dehydrogenase) (IMDH)	93.7	6.0e-114	TC203974	homologue to UP|LEU3_SOLTU (P29696) 3-isopropylmalate dehydrogenase chloroplast precursor (Beta-IPM dehydrogenase) (IMDH)	2.0e-34
HVSMEg0004G13f	AF260565	similar to UP|O04439 (O04439) 3-ketoacyl carrier protein synthase III (EC 2.3.-.	75.99	4.8e-72	TC209121	similar to UP|O04439 (O04439) 3-ketoacyl carrier protein synthase III (EC 2.3.-.	3.7e-74
HVSMEg0004L06f	AW982972	similar to UP|Q8S505 (Q8S505) Acid phosphatase	89.68	8.4e-127	TC207757	similar to UP|Q8S505 (Q8S505) Acid phosphatase	7.7e-94
HVSMEg0004N09f	AW983019	UP|Q93XI6 (Q93XI6) Mitochondrial aldehyde dehydrogenase ALDH2	100	2.5e-122	TC203002	UP|Q8LST6 (Q8LST6) Mitochondrial aldehyde dehydrogenase	1.1e-102
HVSMEg0005A08f	BF623345	UP|THN7_HORVU (Q42838) Thionin BTH7 precursor	99.27	1.2e-135	TC220306	UP|Q9T0P1 (Q9T0P1) Alpha purothionin precursor	3.4e-47
HVSMEg0005A10f	BG343182	similar to UP|Q6Z8N3 (Q6Z8N3) Putative phosphoprotein phosphatase PP7	85.04	5.4e-169	TC194757	similar to UP|Q6Z8N3 (Q6Z8N3) Putative phosphoprotein phosphatase PP7	1.6e-158
HVSMEg0005A20f	BG343192	similar to UP|Q42975 (Q42975) Beta-glucosidase (EC 3.2.1.21)	87.22	1.4e-30	TC220649	similar to UP|Q9AXL6 (Q9AXL6) Beta-glucosidase (Fragment)	2.3e-36
HVSMEg0005B03f	BG343199	similar to UP|Q8H7S6 (Q8H7S6) Putative trehalose-6-phosphate synthase	84.01	6.7e-166	TC220830	similar to UP|Q8H7S6 (Q8H7S6) Putative trehalose-6-phosphate synthase	5.2e-146
HVSMEg0005B06f	BG343202	similar to UP|Q8H557 (Q8H557) Putative ubiquitin C-terminal hydrolase	81.94	1.2e-152	TC202492	similar to UP|Q8H557 (Q8H557) Putative ubiquitin C-terminal hydrolase	1.7e-98
HVSMEg0005B12f	BG343207	UP|Q43379 (Q43379) MAP KINASE	99.19	3.2e-27	TC221301	UP|O81599 (O81599) MAP kinase homolog	1.9e-28
HVSMEg0005E01f	BG343260	UP|SYE_HORVU (Q43768) Glutamyl-tRNA synthetase (Glutamate--tRNA ligase) (GluRS)	100	1.5e-117	TC214494	homologue to UP|SYE_HORVU (Q43768) Glutamyl-tRNA synthetase (Glutamate--tRNA ligase) (GluRS)	8.7e-72
HVSMEg0005E13f	BG343272	weakly similar to UP|Q75IK0 (Q75IK0) Putative o-methyltransferase ZRP4	69	6.5e-174	TC213741	similar to UP|ZRP4_MAIZE (P47917) O-methyltransferase ZRP4 (OMT) (EC 2.1.1.- EC 2.1.1.- EC 2.1.1.-)	5.8e-87
HVSMEg0005G17f[a]	BG343356	UP|PDI_HORVU (P80284) Protein disulfide-isomerase precursor (PDI) (Endosperm protein E-1)	99.61	0.0	AW448800	UP|Q93XQ8 (Q93XQ8) Protein disulfide isomerase 2 precursor (EC 5.3.4.1)	0.0
HVSMEg0005I10f	BG343356	UP|PDI_HORVU (P80284) Protein disulfide-isomerase precursor (PDI) (Endosperm protein E-1)	99.61	0.0	TC206472	UP|Q7FYS2 (Q7FYS2) Protein disulfide isomerase 1 precursor (EC 5.3.4.1 EC 5.3.4.1)	6.4e-137
HVSMEg0005I17f	BG343363	weakly similar to UP|Q8VYI3 (Q8VYI3) At1g76150/T23E18_38	67.28	4.9e-144	TC207546	weakly similar to UP|Q8VYI3 (Q8VYI3) At1g76150/T23E18_38	4.1e-132
HVSMEg0005J05f	BG343374	UP|Q94L27 (Q94L27) Alcohol dehydrogenase	99.47	6.0e-63	TC218888	homologue to UP|Q6K6C1 (Q6K6C1) Alcohol dehydrogenase class III	8.4e-150
HVSMEg0005K03f	BG343392	homologue to UP|Q8RZF3 (Q8RZF3) Putative ketol-acid reductoisomerase	91.04	1.0e-157	TC190386	homologue to UP|Q8RZF3 (Q8RZF3) Putative ketol-acid reductoisomerase	2.8e-148
HVSMEg0005K17f	BG343405	UP|Q8LK43 (Q8LK43) GSK-like kinase	99.73	7.3e-55	TC192583	UP|Q8LK43 (Q8LK43) GSK-like kinase	4.0e-56
HVSMEg0005O14f	BG343492	homologue to UP|O82774 (O82774) Protein phosphatase 2A 55 kDa B regulatory subunit	90.25	4.4e-167	TC218836	homologue to UP|O82774 (O82774) Protein phosphatase 2A 55 kDa B regulatory subunit	2.8e-163
HVSMEg0006A12f	BG414736	homologue to UP|Q8S1Y5 (Q8S1Y5) Putative ubiquitin conjugating enzyme	91.79	5.1e-144	TC221267	homologue to UP|Q8S1Y5 (Q8S1Y5) Putative ubiquitin conjugating enzyme	2.4e-126
HVSMEg0006B08f[a]	BG343672	homologue to GB|BAD18000 serine/threonine protein kinase SAPK4 {Oryza sativa (japonica cultivar-group);}	94.64	7.2e-77	TC218824	homologue to GB|BAD18000 serine/threonine protein kinase SAPK4 {Oryza sativa (japonica cultivar-group);}	5.2e-77
HVSMEg0006D02f	BG343672	homologue to GB|BAD18000 serine/threonine protein kinase SAPK4 {Oryza sativa (japonica cultivar-group);}	94.64	7.2e-77	TC218824	homologue to GB|BAD18000 serine/threonine protein kinase SAPK4 {Oryza sativa (japonica cultivar-group);}	5.2e-77
HVSMEg0006D03f	BG343673	homologue to UP|Q8RVZ9 (Q8RVZ9) Ferredoxin-NADP(H) oxidoreductase	95.39	1.2e-137	TC218505	UP|Q8RVZ9 (Q8RVZ9) Ferredoxin-NADP(H) oxidoreductase	1.0e-111
HVSMEg0006D08f	BG343678	homologue to UP|Q9S711 (Q9S711) ESTs C22657(S0014) (Transmembrane protein kinase)	90.5	1.6e-163	TC226629	homologue to UP|Q9S711 (Q9S711) ESTs C22657(S0014) (Transmembrane protein kinase)	3.3e-64
HVSMEg0006D24f	BG343692	similar to UP|Q8VZX0 (Q8VZX0) Adenylosuccinate-AMP lyase (EC 4.3.2.2)	73.01	5.5e-119	CK156765	similar to GP|17978590 adenylosuccinate-AMP lyase {Vigna unguiculata}	1.0e-89

Gene ID	Gene accession	Putative function Barley database	% Identity	Barley database hit	Wheat database blast	Putative function Wheat database	Wheat database hit
HVSMEg0006E17f	BG343526	homologue to UP|AATC_ORYSA (P37833) Aspartate aminotransferase cytoplasmic (Transaminase A)	95.09	1.0e-156	TC187669	homologue to UP|AATC_ORYSA (P37833) Aspartate aminotransferase cytoplasmic (Transaminase A)	2.2e-149
HVSMEg0006E20f	BG343529	similar to UP|Q9ZS50 (Q9ZS50) Purple acid phosphatase (EC 3.1.3.2 EC 3.1.3.2)	73.51	1.4e-157	TC195530	similar to UP|Q84KZ2 (Q84KZ2) Purple acid phosphatase	2.3e-103
HVSMEg0006F17f	BI948320	similar to UP|Q9AXQ2 (Q9AXQ2) Mitochondrial processing peptidase beta subunit	76.88	8.0e-189	TC204666	similar to UP|Q9AXQ2 (Q9AXQ2) Mitochondrial processing peptidase beta subunit	1.7e-171
HVSMEg0006G05f	BG343558	UP|INO1_HORVU (O65195) Inositol-3-phosphate synthase	99.22	3.3e-161	TC199323	UP|INO1_WHEAT (Q9S7U0) Inositol-3-phosphate synthase (Myo-inositol-1-phosphate synthase) (MI-1-P synthase) (IPS)	4.1e-83
HVSMEg0006G10f	BG343563	homologue to UP|Q8W421 (Q8W421) 26S proteasome regulatory particle triple-A ATPase subunit5a	95.26	3.0e-160	TC187882	homologue to UP|Q8W421 (Q8W421) 26S proteasome regulatory particle triple-A ATPase subunit5a	2.3e-150
HVSMEg0006J07f	BG343724	UP|G3PX_HORVU (P26517) Glyceraldehyde-3-phosphate dehydrogenase	100	4.6e-164	TC205817	UP|G3PX_HORVU (P26517) Glyceraldehyde-3-phosphate dehydrogenase	8.8e-148
HVSMEg0006J20f	unknown	no hit			no hit	no hit	
HVSMEg0006K12f	BG343752	similar to UP|FTHS_SPIOL (P28723) Formate--tetrahydrofolate ligase (Formyltetrahydrofolate synthetase) (FHS) (FTHFS)	86.41	1.5e-160	|TC187818	similar to UP|Q9SPK5 (Q9SPK5) 10-formyltetrahydrofolate synthetase (EC 6.3.4.3)	1.1e-154
HVSMEg0006K18f	BG343758	similar to UP|Q9FZ85 (Q9FZ85) 3-phosphoserine phosphatase	74.55	3.0e-169	TC222407	similar to UP|Q9FZ85 (Q9FZ85) 3-phosphoserine phosphatase	1.4e-155
HVSMEg0006M04f	BG343790	homologue to UP|Q6YZX6 (Q6YZX6) Putative Aconitate hydratase	93.95	2.5e-152	TC203635	homologue to UP|Q6YZX6 (Q6YZX6) Putative Aconitate hydratase	4.2e-131
HVSMEg0006N17f	BG343825	UP|Q94IG2 (Q94IG2) Casein kinase II alpha	99.4	1.5e-149	TC206954	UP|Q94IG2 (Q94IG2) Casein kinase II alpha	5.2e-141
HVSMEg0007A12f	BG343889	similar to UP|Q6J2K7 (Q6J2K7) Protein tyrosine phosphatase	76.79	2.8e-167	TC223696	similar to UP|O82710 (O82710) Protein tyrosine phosphatase (EC 3.1.3.48)	5.1e-110
HVSMEg0007D20f	BG343960	homologue to UP|O24400 (O24400) Cu/Zn superoxide dismutase (EC 1.15.1.1)	95.52	9.3e-148	TC206496	UP|O24400 (O24400) Cu/Zn superoxide dismutase (EC 1.15.1.1)	2.7e-111
HVSMEg0008A01f	AW983075	homologue to UP|Q9ZPJ1 (Q9ZPJ1) S-adenosylmethionine decarboxylase	95.66	4.8e-154	TC190312	UP|Q9ZPJ1 (Q9ZPJ1) S-adenosylmethionine decarboxylase	2.3e-146
HVSMEg0008B09f	BG344201	homologue to UP|Q6Z2M3 (Q6Z2M3) Putative phosphatidate cytidylyltransferase domain-containing protein	92.2	2.9e-107	TC230644	similar to UP|Q94A03 (Q94A03) Putative phosphatidate cytidylyltransferase	5.1e-75
HVSMEg0008B19f	BG344204	UP|GLN2_HORVU (P13564) Glutamine synthetase leaf isozyme chloroplast precursor (Glutamate--ammonia ligase)	99.77	1.6e-67	TC204457	UP|Q6RUJ0 (Q6RUJ0) Glutamine synthetase isoform GSe1	5.0e-95
HVSMEg0008B21f	BG344205	UP|METK_HORVU (P50299) S-adenosylmethionine synthetase 1	99.49	7.9e-101	TC187799	homologue to UP|Q9LGU6 (Q9LGU6) Similar to Oryza sativa S-adenosylmethionine synthetase 1	7.1e-155
HVSMEg0008C18f	BG344214	homologue to UP|Q84P72 (Q84P72) Receptor-like protein kinase-like protein (Fragment)	90.84	6.5e-171	TC208480	homologue to UP|Q852I3 (Q852I3) Gibberellin-induced receptor-like kinase TMK	4.4e-52
HVSMEg0008D08f	AW983113	similar to UP|Q6ZL26 (Q6ZL26) Mitogen activated protein kinase kinase	87.41	4.0e-25	TC211503	similar to UP|Q8LST2 (Q8LST2) Protein kinase	4.9e-42
HVSMEg0008F21f	BG344249	similar to UP|Q9LGS7 (Q9LGS7) Putative cytochrome P450	70.85	2.2e-170	TC193183	similar to UP|Q9LGS7 (Q9LGS7) Putative cytochrome P450	3.1e-138
HVSMEg0008H03f	BG344262	homologue to UP|Q75HJ3 (Q75HJ3) Putative TCP-1/cpn60 chaperonin family protein	92.96	7.9e-90	TC189758	homologue to UP|Q75HJ3 (Q75HJ3) Putative TCP-1/cpn60 chaperonin family protein	1.8e-83
HVSMEg0008I03f	AW983171	homologue to UP|Q8LMR0 (Q8LMR0) Putative phosphoserine aminotransferase	95.07	3.4e-140	TC208876	homologue to UP|Q8LMR0 (Q8LMR0) Putative phosphoserine aminotransferase	1.9e-126
HVSMEg0008I13f	AW983176	similar to UP|Q70AC6 (Q70AC6) Alpha glucosidase II (EC 3.2.1.20)	77	1.7e-153	TC208476	similar to UP|Q70AC6 (Q70AC6) Alpha glucosidase II (EC 3.2.1.20)	1.1e-143
HVSMEg0008I14f	BG344276	UP|FABB_HORVU (P23902) 3-oxoacyl-[acyl-carrier-protein] synthase I chloroplast precursor	100	6.1e-71	TC192206	similar to UP|FABB_ARATH (P52410) 3-oxoacyl-[acyl-carrier-protein] synthase I chloroplast precursor	1.0e-101
HVSMEg0008J16f	AW983189	similar to UP|Q6W0C7 (Q6W0C7) Pto-like serine/threonine kinase	86.25	6.5e-161	TC193005	similar to UP|Q9LX66 (Q9LX66) Receptor protein kinase-like	8.2e-141
HVSMEg0008O01f	AW983242	similar to UP|Q6YZ94 (Q6YZ94) Putative RNA Binding Protein 45	86.34	2.5e-149	TC187747	similar to UP|Q6YZ94 (Q6YZ94) Putative RNA Binding Protein 45	2.5e-141

Gene ID	Gene accession	Putative function Barley database	% Identity	Barley database hit	Wheat database blast	Putative function Wheat database	Wheat database hit
HVSMEg0009C20f	BM816742	UP|UDPG_HORVU (Q43772) UTP--glucose-1-phosphate uridylyltransferase (UDP-glucose pyrophosphorylase) (UDPGP)	100	2.3e-122	TC189820	UP|UDPG_HORVU (Q43772) UTP--glucose-1-phosphate uridylyltransferase (UDP-glucose pyrophosphorylase) (UDPGP)	1.1e-116
HVSMEg0009E19f	BG344447	similar to PIR|F96607 probable clathrin-associated adaptor protein F25P12.96 [imported] - Arabidopsis thaliana	70.05	4.8e-171	TC226901	similar to PIR|F96607 probable clathrin-associated adaptor protein F25P12.96 [imported] - Arabidopsis thaliana	2.2e-150
HVSMEg0009H19f	BG344513	homologue to UP|HBPB_WHEAT (P23923) Transcription factor HBP-1b(c38)	98.79	9.7e-171	TC192147	UP|HBPB_WHEAT (P23923) Transcription factor HBP-1b(c38)	2.2e-107
HVSMEg0009I01f	BG344519	similar to PIR|A84861 probable amine oxidase [imported] - Arabidopsis thaliana {Arabidopsis thaliana;}	75.78	3.6e-165	TC191122	similar to PIR|A84861 probable amine oxidase [imported] - Arabidopsis thaliana {Arabidopsis thaliana;}	6.3e-156
HVSMEg0009L08f	BG344579	homologue to UP|Q9FRB2 (Q9FRB2) Similar to Pisum sativum methylenetetrahydrofolate dehydrogenase (NADP+) (AF030516)	92.33	2.7e-159	TC221026	homologue to UP|Q9FRB2 (Q9FRB2) Similar to Pisum sativum methylenetetrahydrofolate dehydrogenase (NADP+) (AF030516)	5.8e-148
HVSMEg0009L23f	BG344593	similar to GP|4973431 putative aldehyde dehydrogenase OS-ALDH {Oryza sativa subsp. indica}	78.41	2.6e-154	TC208083	similar to UP|Q6H6W9 (Q6H6W9) Putative aldehyde dehydrogenase	5.3e-115
HVSMEg0009P05f	BG344657	homologue to UP|FENT_ORYSA (O23877) Ferredoxin--NADP reductase embryo isozyme chloroplast precursor (FNR)	92.49	3.1e-164	TC206782	homologue to UP|FENT_ORYSA (O23877) Ferredoxin--NADP reductase embryo isozyme chloroplast precursor (FNR)	5.3e-146
HVSMEg0010A06f	AW983267	similar to UP|Q6PW76 (Q6PW76) Delta-1-pyrroline-5-carboxylate synthetase (EC 2.7.2.11 EC 1.2.1.41)	89.02	6.5e-134	TC192632	homologue to UP|Q941T1 (Q941T1) Putative delta l pyrroline-5-carboxylate synthetase	2.6e-77
HVSMEg0010F11f	AW983356	homologue to UP|Q9FRB2 (Q9FRB2) Similar to Pisum sativum methylenetetrahydrofolate dehydrogenase (NADP+) (AF030516)	92.33	5.1e-160	TC221026	homologue to UP|Q9FRB2 (Q9FRB2) Similar to Pisum sativum methylenetetrahydrofolate dehydrogenase (NADP+) (AF030516)	7.2e-148
HVSMEg0010H14f[a]	AW983383	homologue to GB|BAD18000 serine/threonine protein kinase SAPK4 {Oryza sativa (japonica cultivar-group);}	94.64	7.2e-77	TC187966	homologue to UP|Q7XQP4 (Q7XQP4) OSJNBa0084A10.11 protein (Serine/threonine protein kinase SAPK7)	1.4e-97
HVSMEg0010H21f	AW983400	UP|CBP2_HORVU (P08818) Serine carboxypeptidase II precursor (Carboxypeptidase D) (CP-MII)	99.58	8.0e-28	TC190902	homologue to UP|CBP2_HORVU (P08818) Serine carboxypeptidase II precursor (Carboxypeptidase D) (CP-MII)	1.6e-29
HVSMEg0010J10f	AW983424	homologue to UP|Q9SNK3 (Q9SNK3) EST C74302(E30840) corresponds to a region of the predicted gene	91.61	7.6e-152	TC219282	homologue to UP|Q9SNK3 (Q9SNK3) EST C74302(E30840) corresponds to a region of the predicted gene	3.3e-135
HVSMEg0010P04f	AW983525	homologue to UP|Q94JL1 (Q94JL1) Putative CER3	90.33	1.7e-156	TC192306	homologue to UP|Q94JL1 (Q94JL1) Putative CER3	4.2e-142
HVSMEg0010P05f	AW983526	homologue to UP|Q6K9Q5 (Q6K9Q5) Protein phosphatase	94.62	1.5e-171	TC218387	homologue to UP|Q6K9Q5 (Q6K9Q5) Protein phosphatase	1.6e-147
HVSMEg0011B12f	BE060160	homologue to UP|Q8RZJ8 (Q8RZJ8) Similar to protein kinase	90.49	1.3e-61	TC193214	homologue to UP|Q8W3E7 (Q8W3E7) Putative kinase	5.1e-64
HVSMEg0011C03f	BE060168	UP|Q93XI6 (Q93XI6) Mitochondrial aldehyde dehydrogenase ALDH2	100	1.8e-135	TC202875	homologue to UP|Q8LST6 (Q8LST6) Mitochondrial aldehyde dehydrogenase	1.3e-128
HVSMEg0011F04f	BE060065	UP|Q43475 (Q43475) SNF1-related protein kinase (Fragment)	99.59	5.9e-46	NP435396	SNF1-related protein kinase [Triticum aestivum]	2.1e-35
HVSMEg0011G05f	BE060079	homologue to UP|Q6K1T2 (Q6K1T2) Putative lysyl-tRNA synthetase	90.43	1.1e-137	TC213993	homologue to UP|Q6K1T2 (Q6K1T2) Putative lysyl-tRNA synthetase	2.4e-74
HVSMEg0011G23f	BE060096	UP|Q945T7 (Q945T7) Phytochrome C (Fragment)	99.38	8.1e-62	TC194281	UP|Q8VWN1 (Q8VWN1) Phytochrome C	2.6e-129
HVSMEg0011I12f	BE060120	similar to UP|Q93Y60 (Q93Y60) Putative chorismate mutase	85.99	1.0e-121	TC192985	similar to UP|Q93Y60 (Q93Y60) Putative chorismate mutase	4.6e-114
HVSMEg0011M01f	BE060255	UP|Q9M4C7 (Q9M4C7) Allene oxide synthase (EC 4.2.1.92)	99.38	1.8e-124	TC207280	homologue to UP|Q9M4C7 (Q9M4C7) Allene oxide synthase (EC 4.2.1.92)	2.0e-118
HVSMEg0011P06f	AJ295232	similar to PIR|G84590 probable heat shock protein [imported] - Arabidopsis thaliana	80.34	8.5e-73	TC192759	similar to PIR|G84590 probable heat shock protein [imported] - Arabidopsis thaliana {Arabidopsis thaliana;}	9.4e-74
HVSMEg0011P14f	BE060320	UP|SUS1_HORVU (P31922) Sucrose synthase 1 (Sucrose-UDP glucosyltransferase 1)	99.38	5.1e-110	TC187792	homologue to UP|Q9SNK3 (Q9SNK3) EST C74302(E30840) corresponds to a region of the predicted gene	1.6e-103
HVSMEg0012A04f	BE060329	homologue to UP|Q6IWA4 (Q6IWA4) Cycloartenol synthase	93.75	5.4e-123	TC208296	homologue to UP|Q6IWA4 (Q6IWA4) Cycloartenol synthase	2.3e-92
HVSMEg0012B02f	BE060349	homologue to UP|Q8H3L8 (Q8H3L8) FYVE finger-containing phosphoinositide kinase-like	94.21	2.3e-66	TC213976	similar to UP|Q8H3L8 (Q8H3L8) FYVE finger-containing phosphoinositide kinase-like	1.2e-39

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HVSMEg0012D08f	BE060400	UP|Q9M7K3 (Q9M7K3) HAK2	99.48	5.7e-41	TC191934	homologue to UP|Q9M7K3 (Q9M7K3) HAK2	5.8e-39
HVSMEg0012D09f	BE060401	similar to UP|PIMT_WHEAT (Q43209) Protein-L-isoaspartate O-methyltransferase (Protein-beta-aspartate methyltransferase)	72.22	8.9e-101	TC224755	UP|PIMT_WHEAT (Q43209) Protein-L-isoaspartate O-methyltransferase (Protein-beta-aspartate methyltransferase)	1.6e-42
HVSMEg0012D19f	BE060411	homologue to UP|HEM2_HORVU (Q42836) Delta-aminolevulinic acid dehydratase chloroplast precursor (Porphobilinogen synthase)	98.36	1.5e-130	TC218406	homologue to UP|HEM2_HORVU (Q42836) Delta-aminolevulinic acid dehydratase chloroplast precursor (Porphobilinogen synthase)	1.2e-120
HVSMEg0012F21f	BE060460	homologue to UP|Q84P58 (Q84P58) Adenosine kinase-like protein (Fragment)	94.38	5.0e-131	TC219234	homologue to UP|Q84P58 (Q84P58) Adenosine kinase-like protein (Fragment)	8.8e-125
HVSMEg0012F22f	BE060461	homologue to UP|Q6ZHC3 (Q6ZHC3) Putative aspartate-tRNA ligase	91.7	5.7e-137	TC206504	homologue to UP|Q6ZHC3 (Q6ZHC3) Putative aspartate-tRNA ligase	2.8e-131
HVSMEg0012G16f	AW983524	homologue to UP|Q9M7S2 (Q9M7S2) 4-coumarate--CoA ligase 4CL2 (EC 6.2.1.12)	90.94	6.2e-25	TC224497	homologue to UP|Q9M7S2 (Q9M7S2) 4-coumarate--CoA ligase 4CL2 (EC 6.2.1.12)	6.4e-22
HVSMEg0012I03f	BE060512	homologue to UP|Q6K270 (Q6K270) Nodulation receptor kinase-like protein	85.51	2.8e-129	TC194295	similar to UP|Q6K270 (Q6K270) Nodulation receptor kinase-like protein	3.1e-105
HVSMEg0012I12f	BE060521	similar to UP|Q7Y096 (Q7Y096) Putative 3-isopropylmalate dehydrogenase	87.83	3.3e-80	TC192848	similar to UP|LE32_ARATH (P93832) 3-isopropylmalate dehydrogenase 2 chloroplast precursor (Beta-IPM dehydrogenase 2)	1.2e-78
HVSMEg0013C11f	BE060728	homologue to UP|Q8S4W8 (Q8S4W8) Pyruvate decarboxylase	93.75	1.2e-130	TC188783	homologue to UP|Q8S4W8 (Q8S4W8) Pyruvate decarboxylase	5.9e-121
HVSMEg0013D03f	BE060737	homologue to UP|Q6QWQ3 (Q6QWQ3) Fructose 1 6-bisphosphate aldolase (EC 4.1.2.13)	93.02	2.6e-146	TC189637	homologue to UP|Q6QWQ3 (Q6QWQ3) Fructose 1 6-bisphosphate aldolase (EC 4.1.2.13)	8.2e-131
HVSMEg0013D23f	BE060755	similar to UP|Q8GUA6 (Q8GUA6) Phosphatidylinositol 3-kinase	77.87	9.6e-79	TC212869	similar to UP|Q8GUA6 (Q8GUA6) Phosphatidylinositol 3-kinase	8.0e-142
HVSMEg0013F07f	BE060775	UP|O48959 (O48959) Acetyl-coenzyme A carboxylase (EC 6.4.1.2)	99.1	5.2e-31	TC208144	UP|Q41525 (Q41525) Acetyl-CoA carboxylase	2.0e-84
HVSMEg0013F09f[a]	AJ012281	homologue to UP|Q6K1R5 (Q6K1R5) Putative adenosine kinase	94.38	1.5e-177	TC219234	homologue to UP|Q6K1R5 (Q6K1R5) Putative adenosine kinase	1.7e-190
HVSMEg0013F23f	BE060791	similar to UP|Q84V55 (Q84V55) Secretory acid phosphatase	85.55	3.4e-142	CB307037	similar to GP|22202696 putative purple acid phosphatase {Oryza sativa (japonica cultivar-group)}	4.3e-47
HVSMEg0013G19f	BE060801	similar to UP|Q94FS8 (Q94FS8) CaaX processing zinc-metallo endoprotease	77.83	3.0e-130	TC211574	similar to PIR|C85017 probable CAAX prenyl proteinase [imported] - Arabidopsis thaliana {Arabidopsis thaliana;}	7.8e-124
HVSMEg0013H12f	BE060814	UP|CP22_HORVU (P55748) Serine carboxypeptidase II-2 precursor (CP-MII.2) (Fragment) (EC 3.4.16.6)	99.54	2.0e-113	TC192355	homologue to UP|CP22_HORVU (P55748) Serine carboxypeptidase II-2 precursor (CP-MII.2) (Fragment) (EC 3.4.16.6 EC 3.4.16.6)	5.3e-97
HVSMEg0013I07f	BG415492	UP|Q9LW89 (Q9LW89) Adenine phosphoribosyltransferase	100	2.5e-45	TC206512	UP|APT1_WHEAT (Q43199) Adenine phosphoribosyltransferase 1 (APRT) (EC 2.4.2.7 EC 2.4.2.7 EC 2.4.2.7)	5.8e-45
HVSMEg0013J19f	BE060855	UP|LX23_HORVU (Q8GSM2) Lipoxygenase 2.3 chloroplast precursor (LOX2:Hv:3) (EC 1.13.11.12)	100	1.0e-66	TC224576	homologue to UP|LX23_HORVU (Q8GSM2) Lipoxygenase 2.3 chloroplast precursor (LOX2:Hv:3) (EC 1.13.11.12 EC 1.13.11.12)	1.1e-63
HVSMEg0013J22f	BE060858	homologue to UP|ZB14_MAIZE (P42856) 14 kDa zinc-binding protein (Protein kinase C inhibitor) (PKCI)	92.17	3.7e-115	TC207870	homologue to UP|ZB14_MAIZE (P42856) 14 kDa zinc-binding protein (Protein kinase C inhibitor) (PKCI)	1.7e-107
HVSMEg0013L05f	BE060877	similar to UP|Q6ZFX8 (Q6ZFX8) Putative growth regulator	86.73	3.2e-148	TC191869	similar to UP|Q6ZFX8 (Q6ZFX8) Putative growth regulator	1.2e-133
HVSMEg0013N14f	X81394	homologue to UP|Q8GTY8 (Q8GTY8) CDP2_ORYSA Calcium-dependent protein kinase	93.89	1.7e-299	TC220698	homologue to UP|Q8GTY8 (Q8GTY8) CDP2_ORYSA Calcium-dependent protein kinase	7.5e-296
HVSMEg0013P04f	BE060944	UP|Q8GTR5 (Q8GTR5) BZIP transcription factor ZIP1	100	5.4e-169	TC210478	homologue to UP|Q8GTR5 (Q8GTR5) BZIP transcription factor ZIP1	3.3e-161
HVSMEg0015B18f	BE230961	homologue to UP|Q6Z875 (Q6Z875) Putative 26S proteasome regulatory particle triple-A ATPase subunit3	95.26	2.4e-142	TC203672	homologue to UP|Q6Z875 (Q6Z875) Putative 26S proteasome regulatory particle triple-A ATPase subunit3	2.0e-132
HVSMEg0015D01f	BE455799	UP|Q9MAY8 (Q9MAY8) Endo-1 4-beta-glucanase Cel1	99.68	2.8e-124	TC202736	UP|Q9MAY8 (Q9MAY8) Endo-1 4-beta-glucanase Cel1	7.8e-119
HVSMEg0015D05f	BE455801	homologue to UP|Q9AR38 (Q9AR38) Protoporphyrinogen oxidase I	91.75	2.0e-122	AL827014	homologue to GP|13274994 wheat protox-1 {Triticum aestivum}	2.9e-71

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HVSMEg0015E07f	BG344702	homologue to UP|Q9M7S2 (Q9M7S2) 4-coumarate--CoA ligase 4CL2 (EC 6.2.1.12)	90.94	2.2e-135	TC221950	homologue to UP|Q9M7S1 (Q9M7S1) 4-coumarate--CoA ligase 4CL3 (EC 6.2.1.12)	6.7e-98
HVSMEg0015F01f	BE455810	similar to UP|Q6YUU3 (Q6YUU3) Putative leucine-rich repeat transmembrane protein kinase	88.21	1.3e-123	TC208946	similar to UP|Q6YUU3 (Q6YUU3) Putative leucine-rich repeat transmembrane protein kinase	5.8e-116
HVSMEg0015F18f	BE230983	UP|Q7XZK3 (Q7XZK3) Thioredoxin h isoform 1	100	4.2e-137	TC206788	UP|Q8GVD3 (Q8GVD3) Thioredoxin H	4.6e-87
HVSMEg0015G03f	BG344715	UP|Q40058 (Q40058) HSP70 precursor	99.83	1.3e-64	TC220486	UP|Q9SAU8 (Q9SAU8) HSP70	2.3e-125
HVSMEg0015N03f	BE455857	homologue to UP|Q75V56 (Q75V56) Serine/threonine protein kinase SAPK10	96.08	2.6e-36	TC193657	homologue to UP|Q75V57 (Q75V57) Serine/threonine protein kinase SAPK9	6.5e-37
HVSMEg0015N14f	BE231026	homologue to UP|Q6ZIT3 (Q6ZIT3) Putative methionyl aminopeptidase (EC 3.4.11.18)	92.13	6.4e-170	TC191354	homologue to UP|Q6ZIT3 (Q6ZIT3) Putative methionyl aminopeptidase (EC 3.4.11.18)	5.0e-153
HVSMEg0015P21f	BE455877	homologue to UP|Q7F0M8 (Q7F0M8) Putative CRK1 protein(Cdc2-related kinase 1)	93.73	3.1e-32	TC224878	homologue to UP|Q7F0M8 (Q7F0M8) Putative CRK1 protein(Cdc2-related kinase 1)	2.8e-33
HVSMEg0016A14f	BG344779	homologue to UP|Q6ZG77 (Q6ZG77) Putative diaminopimelate decarboxylase	94.75	4.6e-153	TC222141	homologue to UP|Q6ZG77 (Q6ZG77) Putative diaminopimelate decarboxylase	9.1e-116
HVSMEg0016B02f	BG344787	similar to UP|COPD_ORYSA (P49661) Coatomer delta subunit (Delta-coat protein) (Delta-COP) (Archain)	87.11	1.0e-135	CA619384	homologue to GP|7677262 delta-COP {Zea mays}	1.3e-46
HVSMEg0016C06f[a]	BG344787	similar to UP|COPD_ORYSA (P49661) Coatomer delta subunit (Delta-coat protein) (Delta-COP) (Archain)	87.11	1.0e-135	BE446428	similar to UP|COPD_ORYSA (P49661) Coatomer delta subunit (Delta-coat protein) (Delta-COP) (Archain)	1.8e-128
HVSMEg0016D20f	BG344820	homologue to UP|Q6I573 (Q6I573) Putative 1-deoxy-D-xylulose-5-phosphate synthase	91.77	1.4e-133	TC195305	similar to UP|Q6I573 (Q6I573) Putative 1-deoxy-D-xylulose-5-phosphate synthase	1.1e-100
HVSMEg0016E24f	BG344835	homologue to UP|CYSK_WHEAT (P38076) Cysteine synthase (O-acetylserine sulfhydrylase) (O-acetylserine (Thiol)-lyase) (CSase A)	98.15	6.5e-158	TC220004	UP|CYSK_WHEAT (P38076) Cysteine synthase (O-acetylserine sulfhydrylase) (O-acetylserine (Thiol)-lyase) (CSase A)	1.1e-144
HVSMEg0016H07f	BE231083	similar to UP|Q8S8Y8 (Q8S8Y8) Ser/Thr kinase	74.26	2.6e-43	TC214450	homologue to UP|Q7XB94 (Q7XB94) Disease relative signal 1	6.0e-40
HVSMEg0016H13f	BE231086	similar to UP|Q84MU6 (Q84MU6) Putative leucine-rich repeat transmembrane protein kinase	75.68	9.0e-30	CD453086	weakly similar to GP|11244814 serine/threonine protein kinase {Oryza meyeriana}	4.6e-46
HVSMEg0016I12f	BG344863	homologue to UP|Q8LPV1 (Q8LPV1) Acetyl CoA synthetase (Fragment)	90.7	1.4e-94	BQ483947	homologue to GP|4107276 acetyl-CoA synthetase {Solanum tuberosum}	6.3e-51
HVSMEg0016J08f	BE456018	similar to UP|AFC2_ARATH (P51567) Protein kinase AFC2 (EC 2.7.1.-)	83.21	6.4e-125	TC203170	similar to UP|AFC2_ARATH (P51567) Protein kinase AFC2 (EC 2.7.1.- EC 2.7.1.- EC 2.7.1.-)	1.3e-49
HVSMEg0016J10f	BG344871	homologue to UP|O23813 (O23813) Ferredoxin-sulfite reductase precursor (EC 1.8.7.1)	90.92	4.4e-128	TC192747	similar to UP|O23813 (O23813) Ferredoxin-sulfite reductase precursor (EC 1.8.7.1 EC 1.8.7.1)	2.6e-120
HVSMEg0016J24f	BG344874	homologue to UP|Q851Z9 (Q851Z9) Putative dolichyl-phosphate beta-glucosyltransferase	92.02	3.2e-134	CA642045	homologue to GP|27545047 putative dolichyl-phosphate beta-glucosyltransferase {Oryza sativa (japonica cultivar-group)}	1.8e-76
HVSMEg0016O04f	BG344920	homologue to UP|MDHC_MAIZE (Q08062) Malate dehydrogenase cytoplasmic (EC 1.1.1.37)	94.26	3.1e-164	TC187614	homologue to UP|MDHC_MAIZE (Q08062) Malate dehydrogenase cytoplasmic (EC 1.1.1.37 EC 1.1.1.37 EC 1.1.1.-)	2.5e-151
HVSMEg0016P13f	BE231131	homologue to UP|Q75IR7 (Q75IR7) Putative Arginyl-tRNA synthetase	91.69	3.5e-167	TC207625	similar to UP|Q75IR7 (Q75IR7) Putative Arginyl-tRNA synthetase	9.3e-163
HVSMEg0017G18f	BE231181	homologue to UP|Q75HE6 (Q75HE6) Putative methylenetetrahydrofolate reductase	90.57	4.4e-180	TC205083	homologue to UP|Q75HE6 (Q75HE6) Putative methylenetetrahydrofolate reductase	4.2e-166
HVSMEg0017I23f	BE455513	UP|IPYR_HORVD (O23979) Soluble inorganic pyrophosphatase	100	6.7e-55	TC190466	homologue to UP|IPYR_MAIZE (O48556) Soluble inorganic pyrophosphatase	1.1e-92
HVSMEg0017L07f	BE455539	similar to UP|PMM_ARATH (O80840) Probable phosphomannomutase (PMM) (EC 5.4.2.8)	80	2.3e-127	TC222033	similar to UP|PMM_ARATH (O80840) Probable phosphomannomutase (PMM) (EC 5.4.2.8 EC 5.4.2.8)	1.2e-105

Gene ID	Gene accession	Putative function Barley database	% Identity	Barley database hit	Wheat database blast	Putative function Wheat database	Wheat database hit
HVSMEg0017M14f	BE231215	homologue to UP|Q8LP96 (Q8LP96) Moco containing protein (Moco containing protein(OsMCP))	91.94	1.8e-178	TC193475	homologue to UP|Q8LP96 (Q8LP96) Moco containing protein (Moco containing protein(OsMCP))	3.6e-169
HVSMEg0017N01f	BE455560	similar to UP|Q96537 (Q96537) Acyl CoA synthetase (EC 6.2.1.3)	71.14	1.8e-125	TC221991	weakly similar to UP|Q96537 (Q96537) Acyl CoA synthetase (EC 6.2.1.3 EC 6.2.1.3)	7.2e-111
HVSMEg0017N15f	unknown	no hit			no hit	no hit	
HVSMEg0017N19f	BE455569	homologue to UP|Q6Z107 (Q6Z107) Putative receptor protein kinase PERK1	92.5	6.5e-58	TC212016	homologue to UP|Q6Z107 (Q6Z107) Putative receptor protein kinase PERK1	3.4e-107
HVSMEg0018A09f	BG344950	weakly similar to PIR|A40811 myosin-light-chain kinase (EC 2.7.1.117) A - slime mold (Dictyostelium discoideum)	67.8	3.2e-127	TC214382	weakly similar to PIR|G84797 probable protein kinase [imported] - Arabidopsis thaliana {Arabidopsis thaliana;}	2.2e-40
HVSMEg0018B03f	BG366668	similar to UP|PMM_ARATH (O80840) Probable phosphomannomutase (PMM) (EC 5.4.2.8)	80	2.9e-81	TC222033	similar to UP|PMM_ARATH (O80840) Probable phosphomannomutase (PMM) (EC 5.4.2.8 EC 5.4.2.8)	1.5e-61
HVSMEg0018B23f	BG344972	homologue to UP|Q94GI1 (Q94GI1) Clathrin assembly protein AP19-like protein	96.25	6.9e-163	TC202859	homologue to UP|Q94GI1 (Q94GI1) Clathrin assembly protein AP19-like protein	7.0e-146
HVSMEg0018J22f	BE455654	homologue to UP|Q6ZBH5 (Q6ZBH5) Putative hydroxymethylglutaryl coenzyme A synthase	93.1	8.8e-117	TC209454	homologue to UP|Q6ZBH5 (Q6ZBH5) Putative hydroxymethylglutaryl coenzyme A synthase	1.1e-111
HVSMEg0018M07f	BG345120	similar to UP|Q93XC2 (Q93XC2) Papain-like cysteine peptidase XBCP3	71.38	5.6e-134	TC224404	similar to UP|Q93XC2 (Q93XC2) Papain-like cysteine peptidase XBCP3	4.9e-118
HVSMEg0018O21f	BG345161	UP|JI23_HORVU (P32024) 23 kDa jasmonate-induced protein	100	1.2e-132	TC194719	similar to UP|JI23_HORVU (P32024) 23 kDa jasmonate-induced protein	6.6e-101
HVSMEg0019C08f	BE455706	homologue to UP|Q8LAL7 (Q8LAL7) NADH dehydrogenase	94.17	2.1e-138	TC187686	homologue to UP|Q8LAL7 (Q8LAL7) NADH dehydrogenase	5.1e-114
HVSMEg0019C14f	BE455709	similar to UP|Q9S834 (Q9S834) ATP-dependent Clp protease subunit ClpP (NClpP1)	86.76	1.2e-159	TC221564	similar to UP|Q9S834 (Q9S834) ATP-dependent Clp protease subunit ClpP (NClpP1)	4.0e-142
HVSMEg0019D17f	BE456002	similar to GP|29367654 DP TF {Oryza sativa (japonica cultivar-group)}	83.33	0.14	TC215178	homologue to UP|Q9FET1 (Q9FET1) DP protein	3.6e-34
HVSMEg0019F09f[a]	BG299843	similar to UP|DNAA_PSEAE (Q9I7C5) Chromosomal replication initiator protein dnaA	72	1.7e-144	CA500111	similar to GP|29124133 putative leukotriene A-4 hydrolase {Oryza sativa (japonica cultivar-group)}	7.5e-07
HVSMEg0019G12f	BE455732	homologue to UP|Q6Z9A3 (Q6Z9A3) Putative GDP-mannose pyrophosphorylase	93.91	2.9e-165	TC207851	homologue to UP|Q6Z9A3 (Q6Z9A3) Putative GDP-mannose pyrophosphorylase	3.7e-143
HVSMEg0019H10f	BE456042	similar to UP|O64413 (O64413) Auxin-binding protein	84.92	3.5e-158	TC195224	similar to UP|O64413 (O64413) Auxin-binding protein	2.6e-123
HVSMEg0019I01f	BE231278	similar to UP|Q94JI1 (Q94JI1) Putative mitochondrial processing peptidase alpha subuunit mitochondrial recursor	86.9	2.4e-139	TC192042	similar to UP|Q94JI1 (Q94JI1) Putative mitochondrial processing peptidase alpha subuunit mitochondrial recursor	2.2e-132
HVSMEg0019J08f	BE456063	homologue to UP|Q6K988 (Q6K988) Putative casein kinase I	96.1	3.6e-172	TC191693	homologue to UP|Q6K988 (Q6K988) Putative casein kinase I	2.2e-161
HVSMEg0019J13f	BE456068	similar to UP|Q9MAY6 (Q9MAY6) Protein kinase 1	70.27	2.4e-116	TC215361	similar to UP|Q9MAY6 (Q9MAY6) Protein kinase 1	9.1e-54
HVSMEg0019L05f	BI958704	homologue to UP|Q84VG0 (Q84VG0) Putative serine/threonine kinase (Putative calmodulin)	98.65	1.3e-105	TC190416	UP|Q7DLR7 (Q7DLR7) Calmodulin	1.3e-22
HVSMEg0019L10f	BG313507	similar to UP|Q8S340 (Q8S340) Purple acid phosphatase	71.16	1.8e-74	TC229372	weakly similar to UP|Q9LXI4 (Q9LXI4) Purple acid phosphatase (EC 3.1.3.2)	2.0e-106
HVSMEg0019L11f	BE456090	homologue to UP|O04123 (O04123) Calcium-dependent protein kinase (EC 2.7.1.-)	95.99	6.3e-48	TC220698	homologue to UP|Q8GTY8 (Q8GTY8) CDP2_ORYSA Calcium-dependent protein kinase	3.7e-50
HVSMEg0019N09f	BE216074	homologue to GP|15289978 putative cytochrome B5 {Oryza sativa (japonica cultivar-group)}	93.08	6.2e-117	TC189309	homologue to UP|Q94DH6 (Q94DH6) Putative cytochrome B5	1.1e-91
HVSMEg0019O22f	BE455785	homologue to UP|Q8S7U0 (Q8S7U0) Serine/threonine protein phosphatase PP2A-4 catalytic subunit	98.71	6.3e-143	TC220633	homologue to UP|Q8S7U0 (Q8S7U0) Serine/threonine protein phosphatase PP2A-4 catalytic subunit	1.0e-116
HVSMEg0019P02f	BE456128	homologue to UP|Q6ZGC2 (Q6ZGC2) Putative isoleucyl-tRNA synthetase	90.07	3.5e-157	TC199033	similar to UP|Q6ZGC2 (Q6ZGC2) Putative isoleucyl-tRNA synthetase	3.1e-123

Gene ID	Gene accession	Putative function Barley database	% Identity	Barley database hit	Wheat database blast	Putative function Wheat database	Wheat database hit
HVSMEg0019P16f	BE456141	similar to UP|O82668 (O82668) MAP3K beta 1 protein kinase (EC 2.7.1.37)	70.33	6.6e-170	TC194880	similar to UP|O82668 (O82668) MAP3K beta 1 protein kinase (EC 2.7.1.37)	2.0e-133
HVSMEh0080A22f[a]	AAA34292	homologue to UP|Q6WV72 (Q6WV72) Histone H4	98.06	3.5e-87	TC188169	PRF|1101277A.0|224293|110 histone H4. {Triticum aestivum;}	6.6e-115
HVSMEh0080A23f	BE193181	UP|IAA2_HORVU (P13691) Alpha-amylase inhibitor BDAI-I precursor	100	6.8e-99	TC220202	UP|O49956 (O49956) Monomeric alpha-amylase inhibitor precursor	7.5e-44
HVSMEh0080B04f	BE193184	UP|THNA_HORVU (P01545) Alpha-hordothionin precursor (Purothionin II)	100	2.1e-134	TC190797	UP|THN2_WHEAT (P32032) Alpha-2-purothionin precursor	3.6e-108
HVSMEh0080C23f	BE193213	UP|Q84T20 (Q84T20) Endosperm-specific beta-amylase 1	100	2.0e-115	TC206518	UP|AMYB_WHEAT (P93594) Beta-amylase (1 4-alpha-D-glucan maltohydrolase) (EC 3.2.1.2 EC 3.2.1.2)	2.5e-50
HVSMEh0080E03f	BE602174	UP|IAAA_HORVU (P28041) Alpha-amylase/trypsin inhibitor CMa precursor (Chloroform/methanol-soluble protein CMa)	99.31	9.5e-134	TC220117	UP|IA01_WHEAT (P16850) Alpha-amylase/trypsin inhibitor CM1 precursor (Chloroform/methanol-soluble protein CM1)	4.4e-113
HVSMEh0080E17f	BE193243	homologue to UP|Q6H881 (Q6H881) Putative sucrose-phosphate synthase	90.75	1.3e-74	TC223211	similar to UP|Q6H881 (Q6H881) Putative sucrose-phosphate synthase	2.4e-127
HVSMEh0080I11f	unknown	no hit			no hit	no hit	
HVSMEh0080I18f	BE454107	similar to UP|Q84NQ1 (Q84NQ1) Putative leucine-rich repeat transmembrane protein kinase 1	84.81	7.5e-136	TC212770	similar to UP|Q84NQ1 (Q84NQ1) Putative leucine-rich repeat transmembrane protein kinase 1	7.0e-78
HVSMEh0080P15f	BU926883	similar to UP|Q9SWE2 (Q9SWE2) Alanine:glyoxylate aminotransferase 2 homolog	80.23	3.9e-50	TC202854	similar to UP|Q9SWE2 (Q9SWE2) Alanine:glyoxylate aminotransferase 2 homolog	1.8e-51
HVSMEh0081E20f	BE193499	similar to UP|Q6ZL26 (Q6ZL26) Mitogen activated protein kinase kinase	87.41	3.3e-20	TC193912	UP|Q84XZ4 (Q84XZ4) Mitogen-activated protein kinase	2.9e-26
HVSMEh0081G05f	BE193508	UP|PGKY_WHEAT (P12783) Phosphoglycerate kinase cytosolic (EC 2.7.2.3)	99.25	3.8e-156	TC190342	UP|PGKY_WHEAT (P12783) Phosphoglycerate kinase cytosolic (EC 2.7.2.3 EC 2.7.2.3)	1.8e-144
HVSMEh0081G18f	BE193520	similar to SP|P37837 Transaldolase (EC 2.2.1.2). [Human] {Homo sapiens}	71.96	1.2e-26	CA682673	similar to SP|Q9S0X4 Transaldolase (EC 2.2.1.2). {Methylomonas aminofaciens}	5.3e-24
HVSMEh0081H15f	BG368906	UP|THG_HORVU (P20230) Gamma hordothionin	100	4.1e-116	TC190406	UP|THG1_WHEAT (P20158) Gamma-1 purothionin	2.2e-75
HVSMEh0081I17f	BE193543	homologue to UP|Q706H9 (Q706H9) Peptide methionine sulfoxide reductase (EC 1.8.4.6)	96.67	1.0e-136	TC207930	homologue to UP|Q706H9 (Q706H9) Peptide methionine sulfoxide reductase (EC 1.8.4.6)	2.9e-95
HVSMEh0081M04f	BE193575	homologue to GB|CAA75793 sucrose synthase 2 {Hordeum vulgare;}	98.91	1.5e-156	TC219990	homologue to GB|CAA75793 sucrose synthase 2 {Hordeum vulgare ;}	6.9e-147
HVSMEh0081N09f	BG418799	similar to UP|Q6YUU3 (Q6YUU3) Putative leucine-rich repeat transmembrane protein kinase	88.21	4.0e-141	TC208945	homologue to UP|Q6YUU3 (Q6YUU3) Putative leucine-rich repeat transmembrane protein kinase	3.3e-125
HVSMEh0081P09f	X98506	homologue to UP|Q6H798 (Q6H798) Putative acetyl-CoA synthetase	90.93	1.4e-160	TC222800	homologue to UP|Q8LPV1 (Q8LPV1) Acetyl CoA synthetase (Fragment)	2.7e-57
HVSMEh0083D01f	BE193985	UP|Q94IG2 (Q94IG2) Casein kinase II alpha	99.4	1.3e-123	TC206954	UP|Q94IG2 (Q94IG2) Casein kinase II alpha	4.4e-115
HVSMEh0083G04f	BE194013	UP|THNB_HORVU (P21742) Beta-hordothionin precursor	100	1.0e-114	TC220306	UP|Q9T0P1 (Q9T0P1) Alpha purothionin precursor	3.0e-101
HVSMEh0083H21f	BE194026	similar to UP|PDA1_MAIZE (Q43270) Phospholipase D alpha 1 (PLD alpha 1) (Choline phosphatase 1)	89.78	4.9e-114	TC190716	similar to UP|PDA1_ORYSA (Q43007) Phospholipase D alpha 1 precursor (PLD alpha 1) (Choline phosphatase 1	1.3e-99
HVSMEh0083L13f	BE194056	similar to UP|Q6Z9C3 (Q6Z9C3) Putative 6-phosphogluconolactonase	77.46	7.0e-124	TC221159	similar to UP|Q6Z9C3 (Q6Z9C3) Putative 6-phosphogluconolactonase	1.7e-106
HVSMEh0084C12f	BE500187	homologue to UP|Q6UN45 (Q6UN45) Plastidic alpha 1 4-glucan phosphorylase 2 (Fragment) (EC 2.4.1.1)	98.12	3.9e-119	TC208266	UP|Q6UZD6 (Q6UZD6) Plastidic alpha 1 4-glucan phosphorylase (Fragment)	1.1e-126
HVSMEh0084F07f[a]	BF623106	homologue to UP|CLPA_PEA (P35100) ATP-dependent Clp protease ATP-binding subunit clpA homolog chloroplast precursor	90.95	1.4e-170	TC219718	homologue to UP|CLPA_PEA (P35100) ATP-dependent Clp protease ATP-binding subunit clpA homolog chloroplast precursor	6.0e-166
HVSMEh0084G18f	BE194173	similar to UP|Q8H666 (Q8H666) Putative iron superoxide dismutase	84.67	2.9e-95	TC194892	similar to UP|Q8H666 (Q8H666) Putative iron superoxide dismutase	1.6e-111
HVSMEh0084N16f	BF621830	similar to UP|Q94DF2 (Q94DF2) Putative serine/threonine-specific protein kinase	77.4	4.0e-128	TC192669	similar to UP|Q94DF2 (Q94DF2) Putative serine/threonine-specific protein kinase	2.3e-109

Gene ID	Gene accession	Putative function Barley database	% Identity	Barley database hit	Wheat database blast	Putative function Wheat database	Wheat database hit
HVSMEh0085B24f	BG343534	PIR|S24984 glucose-1-phosphate adenylyltransferase - barley {Hordeum vulgare;} (EC 2.7.7.27)	100	1.1e-165	TC188894	PIR|S24984 glucose-1-phosphate adenylyltransferase - barley {Hordeum vulgare;} (EC 2.7.7.27)	2.9e-97
HVSMEh0085D01f	BE194336	UP|CBP1_HORVU (P07519) Serine carboxypeptidase I precursor (Carboxypeptidase C) (CP-MI)	100	0.997	BG262855	homologue to GP|167012 carboxypeptidase I precursor {Hordeum vulgare}	0.36
HVSMEh0085H04f	BI479783	homologue to GB|AAQ23060 heat shock factor RHSF6 {Oryza sativa (japonica cultivar-group);}	92	1.6e-51	TC216378	homologue to GB|AAQ23066 heat shock factor RHSF12 {Oryza sativa (japonica cultivar-group);}	2.0e-31
HVSMEh0085I18f	BE194436	homologue to UP|HMT3_MAIZE (Q9FUM8) Homocysteine S-methyltransferase 3	90.28	3.7e-163	TC221220	homologue to UP|HMT3_MAIZE (Q9FUM8) Homocysteine S-methyltransferase 3 (S-methylmethionine:homocysteine methyltransferase 3)	4.5e-143
HVSMEh0085I24f	BE194442	homologue to UP|Q947S3 (Q947S3) Cinnamyl alcohol dehydrogenase 1a	93.33	3.3e-149	TC202908	homologue to UP|Q947S3 (Q947S3) Cinnamyl alcohol dehydrogenase 1a	1.5e-140
HVSMEh0085M22f	BE194507	similar to UP|Q942N5 (Q942N5) Putative auxin-induced protein	86.79	2.0e-46	TC194676	weakly similar to UP|Q942N5 (Q942N5) Putative auxin-induced protein	1.4e-140
HVSMEh0085O04f	BE194523	UP|DFRA_HORVU (P51106) Dihydroflavonol-4-reductase (DFR) (Dihydrokaempferol 4-reductase)	100	9.2e-161	TC192968	UP|Q84JI1 (Q84JI1) Dihydroflavonol 4-reductase	4.8e-148
HVSMEh0085O13f	BE194532	homologue to UP|SPD1_ORYSA (Q9SMB1) Spermidine synthase 1 (Putrescine aminopropyltransferase 1) (SPDSY 1)	94.16	1.1e-133	TC220400	homologue to UP|SPD1_ORYSA (Q9SMB1) Spermidine synthase 1 (Putrescine aminopropyltransferase 1) (SPDSY 1)	2.7e-124
HVSMEh0086B18f	BE194582	weakly similar to UP|Q8GYW7 (Q8GYW7) Acyl-CoA thioesterase	66.56	9.8e-123	TC222200	weakly similar to UP|Q8GYW7 (Q8GYW7) Acyl-CoA thioesterase (At1g01710)	7.6e-116
HVSMEh0086D18f	BE194612	homologue to UP|Q7F0M8 (Q7F0M8) Putative CRK1 protein(Cdc2-related kinase 1)	93.73	1.7e-127	TC224878	homologue to UP|Q7F0M8 (Q7F0M8) Putative CRK1 protein(Cdc2-related kinase 1)	3.8e-118
HVSMEh0086E10f	unknown	no hit			no hit	no hit	
HVSMEh0086G10f	unknown	no hit			no hit	no hit	
HVSMEh0086L06f	BG415671	similar to PIR|B86465 probable Protein kinase [imported] - Arabidopsis thaliana	86.71	1.2e-98	TC208819	homologue to UP|Q7XZW7 (Q7XZW7) Putative receptor-like protein kinase 1	1.5e-43
HVSMEh0086M08f	BE194753	similar to UP|Q948L3 (Q948L3) Drought inducible 22 kD protein	84.4	1.6e-134	TC204964	similar to UP|Q948L3 (Q948L3) Drought inducible 22 kD protein	4.8e-93
HVSMEh0086M24f	unknown	no hit			no hit	no hit	
HVSMEh0087A14f	BE194812	homologue to UP|Q8W011 (Q8W011) Beta-D-xylosidase	98.71	1.5e-23	TC192869	homologue to UP|Q8W011 (Q8W011) Beta-D-xylosidase	3.6e-25
HVSMEh0087A16f	BG415395	similar to UP|Q7X9L4 (Q7X9L4) Proteinase inhibitor Rgpi9 (Fragment)	80.25	6.8e-108	TC190455	similar to UP|Q7X9L4 (Q7X9L4) Proteinase inhibitor Rgpi9 (Fragment)	5.8e-54
HVSMEh0087B02f	BE194818	homologue to UP|Q9FXT8 (Q9FXT8) 26S proteasome regulatory particle triple-A ATPase subunit4	94.49	5.6e-105	TC220516	UP|Q8W3N8 (Q8W3N8) 26S proteasome regulatory particle triple-A ATPase subunit4b (Fragment)	4.7e-94
HVSMEh0087H08f	BE194893	homologue to UP|Q84U08 (Q84U08) Acetohydroxyacid synthase (Fragment) (EC 4.1.3.18)	98.66	8.3e-103	TC207035	UP|Q84U05 (Q84U05) Acetohydroxyacid synthase (Fragment) (EC 4.1.3.18)	2.8e-96
HVSMEh0088B01f	BE195013	similar to UP|Q6ZL61 (Q6ZL61) Putative tryptophan synthase alpha chain	86.5	3.0e-154	TC196550	similar to UP|Q6ZL61 (Q6ZL61) Putative tryptophan synthase alpha chain	4.3e-98
HVSMEh0088B12f	BE195024	similar to UP|Q9SR15 (Q9SR15) Putative tryptophanyl-tRNA synthetase	80.15	8.2e-115	TC210985	similar to UP|Q9SR15 (Q9SR15) Putative tryptophanyl-tRNA synthetase	1.5e-114
HVSMEh0088D05f[a]	AF271384	similar to UP|Q7Y1J1 (Q7Y1J1) Putative indole-3-glycerol phosphate lyase	77.57	5.8e-71	TC197035	UP|Q7XAK6 (Q7XAK6) Indole synthase	6.6e-69
HVSMEh0088E15f	BE195097	homologue to UP|Q9M639 (Q9M639) Epsilon-COP	94.08	2.9e-130	TC202907	homologue to UP|Q9M639 (Q9M639) Epsilon-COP	4.7e-125
HVSMEh0088E21f	BE195103	weakly similar to UP|Q84ZF7 (Q84ZF7) Putative cytochrome P450 71E1	65.22	1.9e-128	TC195972	weakly similar to UP|Q6XQ14 (Q6XQ14) Cytochrome P450 protein CYP71E	6.8e-108
HVSMEh0088H07f	BE195158	UP|PDI_HORVU (P80284) Protein disulfide-isomerase precursor (PDI) (Endosperm protein E-1)	99.61	1.4e-20	AY544173	UP|Q7FYS2 (Q7FYS2) Protein disulfide isomerase 1 precursor (EC 5.3.4.1 EC 5.3.4.1)	0.0
HVSMEh0088H09f[a]	BG418278	similar to UP|Q6H7H9 (Q6H7H9) Carboxyl-terminal proteinase-like	87.39	5.4e-103	TC203529	similar to UP|Q6H7H9 (Q6H7H9) Carboxyl-terminal proteinase-like	1.2e-9
HVSMEh0088J02f	BE195199	similar to UP|Q6ZII0 (Q6ZII0) Putative cytochrome P450	76.38	1.1e-30	TC229957	similar to UP|Q6ZIH1 (Q6ZIH1) Putative cytochrome P450	4.1e-75
HVSMEh0088J22f	BE195219	UP|ALA2_HORVU (P52894) Alanine aminotransferase 2 (GPT) (Glutamic--pyruvic transaminase 2)	100	2.4e-132	TC205722	homologue to UP|ALA2_HORVU (P52894) Alanine aminotransferase 2 (GPT) (Glutamic--pyruvic transaminase 2)	6.8e-124

Gene ID	Gene accession	Putative function Barley database	% Identity	Barley database hit	Wheat database blast	Putative function Wheat database	Wheat database hit
HVSMEh0088K24f	BE195244	UP|Q42839 (Q42839) Chitinase (EC 3.2.1.14)	99.09	6.2e-78	TC222649	UP|Q8W428 (Q8W428) Chitinase 2	5.9e-46
HVSMEh0088L13f	BE195255	UP|ALEU_HORVU (P05167) Thiol protease aleurain precursor (EC 3.4.22.16)	100	5.0e-164	TC219521	homologue to UP|ALEU_HORVU (P05167) Thiol protease aleurain precursor (EC 3.4.22.16 EC 3.4.22.16)	1.6e-150
HVSMEh0088L19f	BE195261	UP|Q6L799 (Q6L799) Granule bound starch synthase I	100	5.3e-113	TC194520	UP|SSG1_WHEAT (P27736) Granule-bound starch synthase I chloroplast precursor (GBSSI)	2.0e-77
HVSMEh0088N11f	BE195297	UP|ASPR_HORVU (P42210) Phytepsin precursor (Aspartic proteinase) (EC 3.4.23.40)	99.61	7.4e-59	TC220289	similar to UP|ASPR_HORVU (P42210) Phytepsin precursor (Aspartic proteinase) (EC 3.4.23.40 EC 3.4.23.40 EC 3.4.23.-)	8.6e-97
HVSMEh0088O06f	BE195316	homologue to UP|UBC4_WHEAT (P16577) Ubiquitin-conjugating enzyme E2-23 kDa (Ubiquitin-protein ligase)	96.74	3.5e-110	TC192512	UP|UBC4_WHEAT (P16577) Ubiquitin-conjugating enzyme E2-23 kDa (Ubiquitin-protein ligase)	4.4e-100
HVSMEh0088P10f	BE195340	similar to UP|Q7XZW7 (Q7XZW7) Putative receptor-like protein kinase 1	89.34	5.5e-117	TC208819	homologue to UP|Q7XZW7 (Q7XZW7) Putative receptor-like protein kinase 1	6.9e-83
HVSMEh0089A15f	BE195366	weakly similar to GP|13486782 putative wall-associated kinase 1 {Oryza sativa (japonica cultivar-group)}	67.24	2.2e-47	BF484929	weakly similar to GP|15451565 Putative wall-associated kinase 2 {Oryza sativa} [Oryza sativa (japonica cultivar-group)]	1.4e-91
HVSMEh0089E06f	BE195511	homologue to UP|Q9FNU8 (Q9FNU8) 26S proteasome RPT6a subunit	97.62	1.7e-147	TC203755	UP|Q9FNU8 (Q9FNU8) 26S proteasome RPT6a subunit	4.4e-117
HVSMEh0089K14f	BE195444	homologue to UP|Q6ZBX8 (Q6ZBX8) Putative aminopeptidase N	90.04	1.1e-116	TC207130	homologue to UP|Q6ZBX8 (Q6ZBX8) Putative aminopeptidase N	7.8e-113
HVSMEh0090A24f	BE195651	UP|THNB_HORVU (P21742) Beta-hordothionin precursor	100	2.2e-75	TC220306	UP|Q9T0P1 (Q9T0P1) Alpha purothionin precursor	4.9e-65
HVSMEh0090C17f	BE195688	homologue to UP|Q6Z3A4 (Q6Z3A4) Putative acetylornithine aminotransferase	90.87	1.6e-153	TC192300	homologue to UP|Q6Z3A4 (Q6Z3A4) Putative acetylornithine aminotransferase	7.5e-143
HVSMEh0090H10f	BE195788	similar to UP|Q6YU89 (Q6YU89) Putative HECT ubiquitin-protein ligase 3	88.4	5.8e-72	TC221589	similar to UP|Q6YU89 (Q6YU89) Putative HECT ubiquitin-protein ligase 3	2.9e-68
HVSMEh0090J03f	BE195825	UP|METK_HORVU (P50299) S-adenosylmethionine synthetase 1	99.24	1.3e-77	TC187799	homologue to UP|Q9LGU6 (Q9LGU6) Similar to Oryza sativa S-adenosylmethionine synthetase 1	1.2e-121
HVSMEh0090K01f	BE195847	similar to UP|Q9FW92 (Q9FW92) Putative cytochrome P450	83.41	2.2e-53	TC223137	similar to UP|Q9FW92 (Q9FW92) Putative cytochrome P450	4.2e-86
HVSMEh0090L01f	AY075606	similar to UP|Q6L4G6 (Q6L4G6) Putative receptor-like protein kinase	86.24	2.0e-27	TC200365	similar to UP|Q6H7J9 (Q6H7J9) Putative wall-associated kinase 4	2.0e-33
HVSMEh0090M09f	AB029456	UP|Q9LRJ0 (Q9LRJ0) Glucose-6-phosphate dehydrogenase	99.41	0.0	TC206985	UP|Q9LRJ0 (Q9LRJ0) Glucose-6-phosphate dehydrogenase	0.0
HVSMEh0091A04f	BE195973	UP|O48542 (O48542) Peptide transporter	99.48	1.8e-25	TC188171	homologue to UP|Q93VE2 (Q93VE2) Peptide transporter	7.5e-121
HVSMEh0091G17f	BE196120	homologue to UP|Q8GVZ0 (Q8GVZ0) Putative isopentenyl pyrophosphate:dimethyllallyl pyrophosphate isomerase	93.89	1.5e-148	TC218782	homologue to UP|Q8GVZ0 (Q8GVZ0) Putative isopentenyl pyrophosphate:dimethyllallyl pyrophosphate isomerase	8.0e-120
HVSMEh0091K05f	BE196200	homologue to UP|Q8VX48 (Q8VX48) Phosphoglucomutase (Fragment) (EC 5.4.2.2)	97.25	7.7e-140	TC219509	UP|Q8VX48 (Q8VX48) Phosphoglucomutase (Fragment) (EC 5.4.2.2)	7.9e-104
HVSMEh0091N12f	BE196273	homologue to UP|Q8RZJ8 (Q8RZJ8) Similar to protein kinase	90.49	1.8e-149	TC222099	homologue to UP|Q8RZJ8 (Q8RZJ8) Similar to protein kinase	3.0e-135
HVSMEh0091P10f	BE196313	similar to UP|Q8LR33 (Q8LR33) Putative naphthoate synthase	87.12	2.7e-101	TC196069	homologue to UP|Q8LR33 (Q8LR33) Putative naphthoate synthase	7.6e-118
HVSMEh0092B02f	BE196352	UP|Q94IG2 (Q94IG2) Casein kinase II alpha	99.4	1.5e-176	TC206954	UP|Q94IG2 (Q94IG2) Casein kinase II alpha	1.1e-166
HVSMEh0092D03f	BE196400	UP|Q8LK43 (Q8LK43) GSK-like kinase	99.73	2.3e-82	TC192583	UP|Q8LK43 (Q8LK43) GSK-like kinase	2.3e-84
HVSMEh0092H09f	BE196497	UP|TPIS_HORVU (P34937) Triosephosphate isomerase cytosolic (TIM) (EC 5.3.1.1)	99.6	9.6e-39	TC204276	UP|Q9FS79 (Q9FS79) Triosephosphat-isomerase	6.0e-39
HVSMEh0092I04f	BE196513	homologue to UP|Q9ZTW6 (Q9ZTW6) Histidyl-tRNA synthetase (Fragment) (EC 6.1.1.21)	98.88	1.0e-127	TC194579	UP|Q9ZTW6 (Q9ZTW6) Histidyl-tRNA synthetase (Fragment) (EC 6.1.1.21)	1.4e-130
HVSMEh0092J16f	BE196548	UP|Q40025 (Q40025) Beta-glucosidase	99.61	6.7e-135	TC192476	homologue to UP|Q40025 (Q40025) Beta-glucosidase	3.3e-87
HVSMEh0092O11f	BE196656	UP|ALDR_HORVU (P23901) Aldose reductase (AR) (Aldehyde reductase) (EC 1.1.1.21)	100	5.4e-144	TC190761	homologue to UP|ALDR_HORVU (P23901) Aldose reductase (AR) (Aldehyde reductase) (EC 1.1.1.21 EC 1.1.1.21)	1.7e-122
HVSMEh0093H01f	BE454252	similar to UP|Q6H7H9 (Q6H7H9) Carboxyl-terminal proteinase-like	87.39	9.0e-81	TC203890	similar to UP|Q6H7H9 (Q6H7H9) Carboxyl-terminal proteinase-like	9.9e-92

Gene ID	Gene accession	Putative function Barley database	% Identity	Barley database hit	Wheat database blast	Putative function Wheat database	Wheat database hit
HVSMEh0093O16f[a]	BF264363	homologue to UP|Q9SAU8 (Q9SAU8) HSP70	98.61	3.3e-121	TC220486	UP|Q9SAU8 (Q9SAU8) HSP70	1.3e-118
HVSMEh0094B02f	BE454512	UP|METK_HORVU (P50299) S-adenosylmethionine synthetase 1 (Methionine adenosyltransferase 1) (AdoMet synthetase 1)	99.49	2.2e-78	CD491507	GP|17529621 S-adenosylmethionine synthetase {Oryza sativa}	1.7e-58
HVSMEh0094G23f	BE454432	UP|TPIS_HORVU (P34937) Triosephosphate isomerase cytosolic (TIM) (EC 5.3.1.1)	99.6	1.4e-129	TC204276	UP|Q9FS79 (Q9FS79) Triosephosphat-isomerase	3.2e-109
HVSMEh0094G24f	BE454433	UP|Q7PCC5 (Q7PCC5) Putative cysteine protease precursor	100	1.2e-151	TC187806	homologue to UP|Q7PCC5 (Q7PCC5) Putative cysteine protease precursor	2.7e-128
HVSMEh0094I19f	BE454451	homologue to UP|IPYR_HORVD (O23979) Soluble inorganic pyrophosphatase (Pyrophosphate phospho-hydrolase) (PPase)	98.54	4.8e-121	TC190465	homologue to UP|IPYR_HORVD (O23979) Soluble inorganic pyrophosphatase (Pyrophosphate phospho-hydrolase) (PPase)	1.9e-96
HVSMEh0094M16f	BE454625	similar to UP|Q7XGA3 (Q7XGA3) Putative cytosolic tRNA-Ala synthetase	83.16	2.7e-167	TC206768	similar to PIR|D96538 cytosolic tRNA-Ala synthetase [imported] - Arabidopsis thaliana {Arabidopsis thaliana;}	1.5e-161
HVSMEh0095C03f[a]	BE454772	UP|Q6UFY6 (Q6UFY6) Caleosin 2	100	9.6e-150	TC189747	homologue to UP|Q6UFY6 (Q6UFY6) Caleosin 2	2.9e-124
HVSMEh0095F20f	BE454874	similar to UP|Q7XZW7 (Q7XZW7) Putative receptor-like protein kinase 1	89.34	3.3e-124	TC208819	homologue to UP|Q7XZW7 (Q7XZW7) Putative receptor-like protein kinase 1	5.6e-111
HVSMEh0095G06f	BE454713	homologue to GB|BAC10860 putative UMP/CMP kinase {Oryza sativa (japonica cultivar-group);}	95.12	1.5e-163	TC220415	homologue to UP|Q6K7H2 (Q6K7H2) Putative UMP/CMP kinase a	7.9e-153
HVSMEh0095G24f	BE454722	similar to UP|LU1A_LYCPN (O04973) 2-isopropylmalate synthase A	77.76	7.3e-173	TC218551	similar to UP|LU1A_LYCPN (O04973) 2-isopropylmalate synthase A (Alpha-isopropylmalate synthase A) (Alpha-IPM synthetase A)	9.7e-161
HVSMEh0095K23f	BE454522	homologue to emb|X00755.1|OSRRN17S Rice gene for 17S ribosomal RNA	97	1.2e-103	TC189433	similar to UP|Q9LX02 (Q9LX02) ESTs AU082316(E3368)	3.6e-94
HVSMEh0095K24f	BE454746	UP|Q96458 (Q96458) 17 kDa class I small heat shock protein	99.33	4.2e-137	TC220924	UP|HS11_WHEAT (P12810) 16.9 kDa class I heat shock protein (Low molecular weight heat shock protein)	4.2e-89
HVSMEh0095N14f	BE455009	UP|LOX1_HORVU (P29114) Lipoxygenase 1 (EC 1.13.11.12)	100	2.3e-128	TC211286	UP|Q41520 (Q41520) Lipoxygenase (Fragment) (EC 1.13.11.12)	5.2e-69
HVSMEh0096D13f	BE455109	homologue to UP|Q8GTB8 (Q8GTB8) Glutathione transferase F5 (EC 2.5.1.18)	91.55	2.7e-167	TC220488	UP|Q8GTB8 (Q8GTB8) Glutathione transferase F5 (EC 2.5.1.18)	4.6e-131
HVSMEh0096L03f	BJ210163	homologue to UP|Q6K9Q5 (Q6K9Q5) Protein phosphatase	94.62	4.8e-125	TC218387	homologue to UP|Q6K9Q5 (Q6K9Q5) Protein phosphatase	9.8e-129
HVSMEh0096N09f	BE196656	UP|ALDR_HORVU (P23901) Aldose reductase (AR) (Aldehyde reductase) (EC 1.1.1.21)	100	5.4e-144	|TC190761	homologue to UP|ALDR_HORVU (P23901) Aldose reductase (AR) (Aldehyde reductase) (EC 1.1.1.21 EC 1.1.1.21)	1.7e-122
HVSMEh0097A14f	unknown	no hit			no hit	no hit	
HVSMEh0097A22f	BF624657	homologue to UP|Q8RZZ3 (Q8RZZ3) Putative Ser/Thr protein phosphatase	97.69	2.5e-149	TC207988	homologue to UP|Q8RZZ3 (Q8RZZ3) Putative Ser/Thr protein phosphatase	3.1e-122
HVSMEh0097K07f	U67422	homologue to PIR|T04108 receptor kinase homolog CRINKLY4 - maize {Zea mays;} (EC 2.7.1.-)	93.89	1.8e-92	CK210671	homologue to GP|15721862 CR4 {Oryza sativa}	1.6e-73
HVSMEh0098B03f	BE602205	homologue to UP|G6PI_MAIZE (P49105) Glucose-6-phosphate isomerase cytosolic (GPI) (Phosphoglucose isomerase) (PGI)	92.77	5.1e-107	TC203688	homologue to UP|G6PI_MAIZE (P49105) Glucose-6-phosphate isomerase cytosolic (GPI) (Phosphoglucose isomerase) (PGI)	1.7e-105
HVSMEh0098B13f[a]	AF271384	similar to UP|Q7Y1J1 (Q7Y1J1) Putative indole-3-glycerol phosphate lyase	77.57	5.8e-71	TC197035	UP|Q7XAK6 (Q7XAK6) Indole synthase	6.6e-69
HVSMEh0098G14f	BE601555	homologue to UP|Q6H881 (Q6H881) Putative sucrose-phosphate synthase	90.75	3.4e-20	TC204185	homologue to UP|Q6SXU0 (Q6SXU0) Sucrose-phosphate synthase (EC 2.4.1.14)	2.8e-111
HVSMEh0098I12f	BE601566	homologue to UP|O22664 (O22664) Cytosolic heat shock 70 protein	92.95	3.8e-139	TC220486	UP|Q9SAU8 (Q9SAU8) HSP70	8.3e-98
HVSMEh0098P19f	BE602463	similar to UP|Q9AVE7 (Q9AVE7) Zeaxanthin epoxidase	89.4	1.9e-110	TC222807	similar to UP|Q9AVE7 (Q9AVE7) Zeaxanthin epoxidase	4.0e-105
HVSMEh0099C03f	BE601667	homologue to UP|Q6L5C4 (Q6L5C4) Protein phosphatase 2C	90.62	5.0e-31	BU100208	homologue to GP|20146110 protein phosphatase 2C {Mesembryanthemum crystallinum}	2.0e-32
HVSMEh0099E06f[a]	BG343905	similar to UP|Q6IV73 (Q6IV73) Protein phosphatase 2C	86.82	3.5e-78	TC188688	similar to UP|Q6IV73 (Q6IV73) Protein phosphatase 2C	2.8e-70
HVSMEh0099G05f	BE601741	GB|AAA34124 pentameric polyubiquitin {Nicotiana sylvestris;}	100	1.4e-83	TC190051	UP|Q40641 (Q40641) Polyubiquitin	5.0e-87

Gene ID	Gene accession	Putative function Barley database	% Identity	Barley database hit	Wheat database blast	Putative function Wheat database	Wheat database hit
HVSMEh0099G07f	BE601743	homologue to UP|Q9SE42 (Q9SE42) D-ribulose-5-phosphate 3-epimerase (EC 5.1.3.1)	91.63	2.4e-97	TC202744	homologue to UP|Q9SE42 (Q9SE42) D-ribulose-5-phosphate 3-epimerase (EC 5.1.3.1)	1.9e-77
HVSMEh0099G19f	BE601754	UP|Q40025 (Q40025) Beta-glucosidase	99.61	4.9e-106	TC192476	homologue to UP|Q40025 (Q40025) Beta-glucosidase	4.6e-96
HVSMEh0099J10f	BE602521	homologue to UP|Q9FUJ7 (Q9FUJ7) Citrate synthase	94.49	1.3e-156	TC203564	homologue to UP|Q9FUJ7 (Q9FUJ7) Citrate synthase	6.0e-144
HVSMEh0099N09f	BE601871	homologue to UP|TBP2_WHEAT (Q02879) TATA-box binding protein 2 (TATA-box factor 2) (TATA binding factor 2)	97.97	2.6e-124	TC208275	UP|TBP2_WHEAT (Q02879) TATA-box binding protein 2 (TATA-box factor 2) (TATA binding factor 2)	1.9e-85
HVSMEh0099O01f	BE601878	weakly similar to UP|Q39807 (Q39807) Protease inhibitor	61.19	4.0e-44	TC207933	weakly similar to UP|Q39807 (Q39807) Protease inhibitor	5.3e-59
HVSMEh0099O18f[a]	HVIAM1	UP|IAA1_HORVU (P16968) Alpha-amylase inhibitor BMAI-1 precursor (Allergen Hor v 1) (Alpha-amylase flour inhibitor)	100	5.5e-126	BE427571	homologue to PIR|T06517 alpha-amylase inhibitor Ima1 precursor monomeric - wheat	0.12
HVSMEh0100J15f	BE602887	similar to UP|Q6ZGW6 (Q6ZGW6) Putative delta-12 oleate desaturase	80.77	2.3e-107	CA641477	homologue to GP|20520624 fatty acid desaturase 2 {Brassica rapa}	4.2e-41
HVSMEh0100J16f	BE601967	homologue to UP|Q8H8D8 (Q8H8D8) Glutathione S-transferase GSTF15	90.58	3.9e-24	TC207575	homologue to UP|Q8GTB8 (Q8GTB8) Glutathione transferase F5 (EC 2.5.1.18)	4.7e-26
HVSMEh0100J22f[a]	BG418805	homologue to UP|FKB7_WHEAT (Q43207) 70 kDa peptidylprolyl isomerase (Peptidyl-prolyl cis-trans isomerase) (PPIase) (Rotamase)	97.32	2.2e-106	TC220854	homologue to UP|FKB7_WHEAT (Q43207) 70 kDa peptidylprolyl isomerase (Peptidyl-prolyl cis-trans isomerase) (PPIase) (Rotamase)	1.7e-88
HVSMEh0100L01f	BE602892	homologue to UP|FKB7_WHEAT (Q43207) 70 kDa peptidylprolyl isomerase (Peptidyl-prolyl cis-trans isomerase) (PPIase) (Rotamase)	97.32	4.7e-108	TC220854	homologue to UP|FKB7_WHEAT (Q43207) 70 kDa peptidylprolyl isomerase (Peptidyl-prolyl cis-trans isomerase) (PPIase) (Rotamase)	9.7e-99
HVSMEh0100L12f[a]	BG418805	homologue to UP|FKB7_WHEAT (Q43207) 70 kDa peptidylprolyl isomerase (Peptidyl-prolyl cis-trans isomerase) (PPIase) (Rotamase)	97.32	2.2e-106	TC220854	homologue to UP|FKB7_WHEAT (Q43207) 70 kDa peptidylprolyl isomerase (Peptidyl-prolyl cis-trans isomerase) (PPIase) (Rotamase)	1.7e-88
HVSMEh0100N24f	BE601995	homologue to UP|Q43638 (Q43638) Heat-shock protein precursor	97.67	7.6e-149	TC206202	homologue to UP|Q43638 (Q43638) Heat-shock protein precursor	1.9e-139
HVSMEh0101A21f	BE602672	UP|CHI1_HORVU (P11955) 26 kDa endochitinase 1 precursor (EC 3.2.1.14)	99.37	1.4e-119	TC222649	UP|Q8W428 (Q8W428) Chitinase 2	8.7e-87
HVSMEh0101C23f	BE602696	similar to UP|Q6Z5P2 (Q6Z5P2) Putative endo-beta-1 4-glucanase	79.59	3.6e-113	CK208903	similar to PIR|T01108 cellulase (EC 3.2.1.4) T21L14.7 - Arabidopsis thaliana	5.7e-40
HVSMEh0101D22f	BE602970	homologue to PIR|S56639 ribosomal protein S6 kinase homolog (clone Aspk11) - oat {Avena sativa;}	92.9	2.9e-105	TC222103	homologue to PIR|S56639 ribosomal protein S6 kinase homolog (clone Aspk11) - oat {Avena sativa;}	4.9e-100
HVSMEh0101E17f	BE602714	UP|Q7XJ80 (Q7XJ80) Cytosolic heat shock protein 90	100	4.6e-117		UP|Q7XJ80 (Q7XJ80) Cytosolic heat shock protein 90	7.5e-118
HVSMEh0101F02f	unknown	no hit			no hit	no hit	
HVSMEh0102A01f	BE602008	homologue to UP|Q84V24 (Q84V24) Aspartate aminotransferase (Fragment)	91.93	7.4e-110	TC207382	homologue to UP|Q84V24 (Q84V24) Aspartate aminotransferase (Fragment)	1.4e-102
HVSMEh0102A08f	BE602015	homologue to UP|UBC7_WHEAT (P25868) Ubiquitin-conjugating enzyme E2 7 (Ubiquitin-protein ligase 7)	98.78	4.8e-158	TC187673	homologue to UP|UBC7_WHEAT (P25868) Ubiquitin-conjugating enzyme E2 7 (Ubiquitin-protein ligase 7)	2.0e-148
HVSMEh0102A09f	BE602016	homologue to UP|Q8RUU6 (Q8RUU6) Pyruvate decarboxylase	91.75	3.1e-108	TC188783	homologue to UP|Q8S4W8 (Q8S4W8) Pyruvate decarboxylase	1.5e-64
HVSMEh0102B06f	BE603119	similar to UP|Q94DF2 (Q94DF2) Putative serine/threonine-specific protein kinase	82.8	7.9e-36	TC212570	similar to UP|Q84QD9 (Q84QD9) Avr9/Cf-9 rapidly elicited protein 264	1.3e-36
HVSMEh0102D20f	BE603155	homologue to UP|Q6IUP7 (Q6IUP7) Putative pyruvate kinase (EC 2.7.1.40)	90.71	5.7e-50	TC203150	homologue to UP|Q6IUP7 (Q6IUP7) Putative pyruvate kinase (EC 2.7.1.40)	1.1e-53
HVSMEh0102G08f	HVACXPII1	UP|CP21_HORVU (P55747) Serine carboxypeptidase II-1 precursor (CP-MII.1) (Fragment) (EC 3.4.16.6)	100	8.3e-274	TC208113	homologue to UP|CP21_HORVU (P55747) Serine carboxypeptidase II-1 precursor (CP-MII.1) (Fragment) (EC 3.4.16.6)	7.0e-241
HVSMEh0102K13f[a]	BE060096	UP|Q945T7 (Q945T7) Phytochrome C (Fragment)	99.88	1.3e-137	TC194281	UP|Q8VWN1 (Q8VWN1) Phytochrome C	4.3e-129
HVSMEh0102L08f	BE603237	similar to UP|Q8S8Z0 (Q8S8Z0) Protein phosphatase 2C	88.17	2.7e-114	BU100208	homologue to GP|20146110 protein phosphatase 2C {Mesembryanthemum crystallinum}	1.1e-86
HVSMEh0102M13f	BE602159	similar to UP|Q6ZD84 (Q6ZD84) Putative P450	76.19	5.8e-57	TC210944	UP|Q8S9E6 (Q8S9E6) P450.complete	3.7e-52

Gene ID	Gene accession	Putative function Barley database	% Identity	Barley database hit	Wheat database blast	Putative function Wheat database	Wheat database hit
HVSMEh0102O04f	BE602172	homologue to UP|Q6YUH4 (Q6YUH4) Putative ethylene-inducible protein	95.83	8.4e-156	TC188999	homologue to UP|Q6YUH4 (Q6YUH4) Putative ethylene-inducible protein	9.1e-135
no match_redo	BE438154	homologue to GB|BAC10351 unknown protein {Oryza sativa (japonica cultivar-group);}	91.12	1.2e-148	TC221926	homologue to GB|BAC10351 unknown protein {Oryza sativa (japonica cultivar-group);}	7.2e-136
pBTag	TC147149	UP|Q43487 (Q43487) Oxalate oxidase-like protein or germin-like protein (Germin-like 8) (Germin-like 12)	100	1.7e-158	AJ237942	UP|Q9SM34 (Q9SM34) Germin-like protein precursor	7.9e-161
pox381	TC140187	UP|Q40068 (Q40068) Peroxidase (EC 1.11.1.7)	100	6.0e-114	X56011	UP|Q43212 (Q43212) Peroxidase precursor (EC 1.11.1.7 EC 1.11.1.7)	6.6e-275
RTL1	At5g13910	AP2/EREBP-like transcription factor LEAFY PETIOLE					
SFR001.A02F990616	BE437186	UP|Q9FS11 (Q9FS11) Vacuolar proton-ATPase	100	4.8e-94	TC206232	UP|Q9FS11 (Q9FS11) Vacuolar proton-ATPase	5.8e-88
SFR001.A11F990616	BE437299	similar to UP|Q8RX85 (Q8RX85) AT3g17750/MIG5_4	87.93	8.5e-235	TC229181	similar to PIR|B96761 probable protein kinase T9L24.36 [imported] - Arabidopsis thaliana {Arabidopsis thaliana;}	1.4e-61
SFR001.B01F990616	BE437301	similar to GP|20146351 putative myosin heavy chain {Oryza sativa (japonica cultivar-group)}	76	1.7e-95	BE585797	similar to GP|20146351 putative myosin heavy chain {Oryza sativa (japonica cultivar-group)}	1.1e-83
SFR001.D01F990616	BE437324	UP|Q8L3P8 (Q8L3P8) Stem rust resistance protein Rpg1 (Barley stem rust resistance protein)	100	2.4e-26	TC208420	similar to UP|Q8H841 (Q8H841) Putative receptor-like protein kinase	1.3e-156
SFR001.D02F990616	BE437325	similar to UP|UCRH_SOLTU (P48504) Ubiquinol-cytochrome C reductase complex 7.8 kDa protein (Mitochondrial hinge protein)	71.64	1.7e-67	TC221972	similar to UP|UCRH_SOLTU (P48504) Ubiquinol-cytochrome C reductase complex 7.8 kDa protein (Mitochondrial hinge protein)	1.7e-55
SFR001.E06F990616	BE437340	homologue to UP|Q6X4A2 (Q6X4A2) CIPK-like protein	91.98	1.6e-130	TC224272	homologue to UP|Q6X4A2 (Q6X4A2) CIPK-like protein	8.0e-50
SFR001.F10F990616	BE437356	weakly similar to UP|Q8SBC3 (Q8SBC3) Protein phosphatase 2C (Fragment)	69.84	1.5e-134	TC223519	similar to UP|Q7XW27 (Q7XW27) OSJNBb0062H02.4 protein	3.8e-118
SFR001.G01F990616	BE437359	similar to UP|Q7G6E9 (Q7G6E9) Putative aldose 1-epimerase-like protein	88.69	5.5e-96	TC207584	similar to UP|Q7G6E9 (Q7G6E9) Putative aldose 1-epimerase-like protein	1.6e-89
SFR001.G07F990616	BE437365	similar to UP|O22105 (O22105) Squalene synthase	87.27	5.4e-165	TC208927	similar to UP|O22106 (O22106) Squalene synthase (EC 2.5.1.21)	5.2e-141
SFR002.A05F990618	BE437387	homologue to UP|Q6ZL42 (Q6ZL42) Putative histone H2A	97.76	3.6e-96	TC206380	UP|Q43312 (Q43312) Protein H2A	9.4e-45
SFR002.A11F990715	BE437393	similar to UP|Q8L4G7 (Q8L4G7) Putative formylglycinamide ribonucleotide amidotransferase	89.6	4.6e-94	TC213282	similar to UP|Q8L4G7 (Q8L4G7) Putative formylglycinamide ribonucleotide amidotransferase	1.1e-44
SFR002.C12F990618	BE437192	similar to UP|Q7Y0V3 (Q7Y0V3) Actin filament bundling protein P-115-ABP	70	3.2e-120	TC209933	weakly similar to UP|Q7Y0V3 (Q7Y0V3) Actin filament bundling protein P-115-ABP	3.9e-105
SFR002.D07F990715	BE437199	homologue to UP|Q7F280 (Q7F280) NADP-specific isocitrate dehydrogenase (EC 1.1.1.42)	94.13	4.6e-111	TC188690	homologue to UP|Q7F280 (Q7F280) NADP-specific isocitrate dehydrogenase (EC 1.1.1.42)	3.6e-98
SFR002.F11F990618	BE437226	weakly similar to UP|Q8H8W2 (Q8H8W2) Putative receptor-like protein kinase	67.65	2.3e-126	TC187916	weakly similar to UP|Q8LN27 (Q8LN27) Putative receptor-like protein kinase	1.3e-112
SFR002.G07F990618	BE437234	homologue to UP|Q9ZPJ1 (Q9ZPJ1) S-adenosylmethionine decarboxylase	95.66	3.3e-77	TC190312	UP|Q9ZPJ1 (Q9ZPJ1) S-adenosylmethionine decarboxylase	2.1e-62
SFR002.H09F990618	BE437247	similar to PIR|T06420 phosphoinositide-specific phospholipase C (EC 3.1.4.-) plasma membrane-associated - soybean	71.43	8.2e-90	TC209325	similar to UP|Q75IL8 (Q75IL8) Putative phosphatidylinositol-specific phospholipase C (EC 3.1.4.- EC 3.1.4.-)	6.6e-13
SFR003.A06F990621	BE437255	UP|Q945R5 (Q945R5) Ascorbate peroxidase	100	9.6e-105	TC218869	UP|Q945R5 (Q945R5) Ascorbate peroxidase	2.1e-79
SFR003.A11F990621	BE437260	homologue to UP|Q9ZWJ2 (Q9ZWJ2) Glyoxalase I	92.41	9.9e-136	TC190366	homologue to UP|Q9ZWJ2 (Q9ZWJ2) Glyoxalase I	1.9e-132
SFR003.A12F990621	BE437261	UP|Q8H1L9 (Q8H1L9) Actin	100	6.3e-24	TC203904	UP|Q75LK6 (Q75LK6) Actin	6.5e-27
SFR003.C02F990621	BE437275	homologue to UP|Q84ZC0 (Q84ZC0) Putative vacuolar ATP synthase subunit H	91.57	8.4e-92	TC191290	homologue to UP|Q84ZC0 (Q84ZC0) Putative vacuolar ATP synthase subunit H	1.5e-77
SFR003.D04F990621	BE437288	UP|Q7XJ80 (Q7XJ80) Cytosolic heat shock protein 90	100	4.7e-98	TC187638	UP|Q7XJ80 (Q7XJ80) Cytosolic heat shock protein 90	8.5e-76

Gene ID	Gene accession	Putative function Barley database	% Identity	Barley database hit	Wheat database blast	Putative function Wheat database	Wheat database hit
SFR003.D07F990621	BE437291	similar to GB|BAC22425 putative nucleolar protein {Oryza sativa (japonica cultivar-group);}	87.2	4.3e-67	TC205731	similar to GB|BAC22425 putative nucleolar protein {Oryza sativa (japonica cultivar-group);}	2.1e-68
SFR003.E02F990621	BE437418	UP|O22575 (O22575) Glycine decarboxylase P subunit (EC 1.4.4.2)	99.37	2.0e-95	TC220061	UP|O22575 (O22575) Glycine decarboxylase P subunit (EC 1.4.4.2)	6.5e-94
SFR003.E10F990621	BE437424	UP|Q8GTR5 (Q8GTR5) BZIP transcription factor ZIP1	100	6.3e-130	TC210478	homologue to UP|Q8GTR5 (Q8GTR5) BZIP transcription factor ZIP1	1.0e-125
SFR003.G02F990621	BE437437	UP|Q43379 (Q43379) MAP KINASE	99.19	9.2e-96	TC221301	UP|Q43379 (Q43379) MAP KINASE	1.6e-88
SFR003.H05F990621	BE437451	GP|11990901 ribulose-1 5-bisphosphate carboxylase/oxygenase small subunit {Triticum aestivum}	99.12	1.6e-39	TC219744	UP|Q9FRZ2 (Q9FRZ2) Ribulose-1 5-bisphosphate carboxylase/oxygenase small subunit	1.4e-39
SFR004.A02F990621	BE437471	homologue to UP|Q7SIC9 (Q7SIC9) Transferase	90.08	8.8e-114	TC218390	similar to UP|Q9FPB6 (Q9FPB6) Putative transketolase	4.1e-106
SFR004.A07F990621	BE437465	similar to UP|Q75I52 (Q75I52) Expressed protein	86.71	3.8e-122	TC193579	similar to UP|Q75I52 (Q75I52) Expressed protein	4.2e-114
SFR004.C02F990621	unknown	no hit			no hit	no hit	
SFR004.C06F990621	BE437488	homologue to GP|18461184 putative katanin {Oryza sativa (japonica cultivar-group)}	93.67	4.1e-116	TC211288	homologue to UP|Q7XXR9 (Q7XXR9) Katanin	6.8e-57
SFR004.C09F990621	BE437491	UP|Q70YJ0 (Q70YJ0) Putative MAP kinase	99.31	4.1e-39	TC225292	homologue to UP|Q8S2I8 (Q8S2I8) MAP kinase-like protein	5.3e-53
SFR004.D01F990621	BE437495	homologue to UP|Q75HX3 (Q75HX3) Putative soluble inorganic pyrophosphatase	90.86	1.8e-111	TC190470	homologue to UP|Q75HX3 (Q75HX3) Putative soluble inorganic pyrophosphatase	9.1e-60
SFR004.D06F990621	BE437499	homologue to UP|P2A3_ORYSA (Q9XGT7) Serine/threonine protein phosphatase PP2A-3 catalytic subunit (EC 3.1.3.16)	97.36	2.5e-144	TC210875	homologue to UP|P2A3_ORYSA (Q9XGT7) Serine/threonine protein phosphatase PP2A-3 catalytic subunit (EC 3.1.3.16 EC 3.1.3.16)	9.7e-130
SFR004.E07F990621	BE437512	UP|Q8H1V3 (Q8H1V3) Hypersensitive-induced reaction protein 1	100	1.6e-65	TC205533	UP|Q8H1V3 (Q8H1V3) Hypersensitive-induced reaction protein 1	1.6e-53
SFR004.E08F990621	BE437513	UP|Q9SME4 (Q9SME4) Glutathione peroxidase-like protein GPX54Hv	100	1.4e-119	TC202747	UP|Q6UQ05 (Q6UQ05) Cytosolic glutathione peroxidase	2.3e-102
SFR004.E09F990621	BE437502	homologue to UP|Q40000 (Q40000) Mg-chelatase subunit (Fragment) (EC 4.99.1.-)	97.26	3.0e-113	TC188313	homologue to UP|Q40000 (Q40000) Mg-chelatase subunit (Fragment) (EC 4.99.1.-)	7.6e-101
SFR004.E12F990621	BE437517	UP|Q7XJ26 (Q7XJ26) Iron/ascorbate-dependent oxidoreductase	100	8.0e-154	TC189998	homologue to UP|Q7XJ26 (Q7XJ26) Iron/ascorbate-dependent oxidoreductase	1.7e-109
SFR004.F01F990621	BE437518	similar to GB|AAP13377 At5g10480 {Arabidopsis thaliana;} protein tyrosine phosphatase-like	77.36	1.5e-135	TC190290	similar to GB|AAP13377 At5g10480 {Arabidopsis thaliana;} protein tyrosine phosphatase-like	7.7e-105
SFR004.G11F990621	BE437539	homologue to UP|RL11_MEDSA (P46287) 60S ribosomal protein L11 (L5)	93.64	9.4e-153	TC187697	homologue to UP|RL11_MEDSA (P46287) 60S ribosomal protein L11 (L5)	2.5e-136
SFR004.H07F990621	BE437546	homologue to UP|O23802 (O23802) Plastid omega-3 fatty acid desaturase (Fragment)	98.73	6.5e-75	TC211303	UP|O23802 (O23802) Plastid omega-3 fatty acid desaturase (Fragment)	1.7e-64
SFR005.B01F990701	BE437563	homologue to UP|O04186 (O04186) Fd-GOGAT protein (Fragment)	93.65	3.8e-107	TC218941	homologue to UP|O04186 (O04186) Fd-GOGAT protein (Fragment)	3.4e-98
SFR005.C04F990715	BE437576	similar to UP|Q7EZ29 (Q7EZ29) Putative MAP4 kinase	79.06	1.9e-125	CK206621	similar to GP|23307577 putative serine/threonine protein kinase {Oryza sativa (japonica cultivar-group)}	8.2e-101
SFR005.D10F990624	BE437593	homologue to UP|Q8LH96 (Q8LH96) Putative phospho-2-dehydro-3-deoxyheptonate aldolase 1 chloroplast	90	1.1e-141	TC220398	homologue to UP|Q8LH96 (Q8LH96) Putative phospho-2-dehydro-3-deoxyheptonate aldolase 1 chloroplast	2.4e-130
SFR005.E01F990722	BE438172	similar to UP|FUM1_ARATH (P93033) Fumarate hydratase 1 mitochondrial precursor (Fumarase 1)	87.5	1.4e-148	TC206879	similar to UP|FUM1_ARATH (P93033) Fumarate hydratase 1 mitochondrial precursor (Fumarase 1)	4.1e-142
SFR005.E05F990701	BE437599	UP|EF1A_WHEAT (Q03033) Elongation factor 1-alpha (EF-1-alpha)	100	6.4e-103	TC189449	UP|EF1A_WHEAT (Q03033) Elongation factor 1-alpha (EF-1-alpha)	1.9e-91
SFR005.F09F990624	BE437615	homologue to UP|P93852 (P93852) Endosperm C-24 sterol methyltransferase (EC 2.1.1.41)	93.9	5.1e-86	TC192488	homologue to UP|P93852 (P93852) Endosperm C-24 sterol methyltransferase (EC 2.1.1.41)	7.5e-56
SFR005.G03F990705	BE437621	similar to UP|Q9FE02 (Q9FE02) Cytochrome c oxidase subunit 6b (OSJNBa0029H02.1 protein) (OSJNBa0067K08.22 protein)	88.31	1.0e-87	TC202813	similar to UP|Q9FE02 (Q9FE02) Cytochrome c oxidase subunit 6b (OSJNBa0029H02.1 protein) (OSJNBa0067K08.22 protein)	3.1e-75

Gene ID	Gene accession	Putative function Barley database	% Identity	Barley database hit	Wheat database blast	Putative function Wheat database	Wheat database hit
SFR005.G08F990705	BE437626	similar to UP|Q6H527 (Q6H527) Putative sds22+	88.54	8.3e-117	TC188107	similar to UP|Q6H527 (Q6H527) Putative sds22+	7.3e-110
SFR005.H08F990623	BE437638	homologue to UP|Q94GW8 (Q94GW8) Putative kinase	92.81	3.0e-100	TC224772	homologue to UP|P93520 (P93520) Calcium/calmodulin-dependent protein kinase homolog|CaM kinase homolog|MCK1 protein	1.3e-20
SFR006.A04F990622	BE437646	similar to UP|Q84PC8 (Q84PC8) Protein kinase-like protein (Fragment)	83.93	5.2e-108	TC222871	similar to UP|Q84PC8 (Q84PC8) Protein kinase-like protein (Fragment)	8.2e-32
SFR006.B02F990621	BE437656	similar to UP|Q8RUD7 (Q8RUD7) Similar to protein kinase AtSIK (P0485B12.21 protein)	86.95	5.8e-139	TC197765	similar to UP|Q8RUD7 (Q8RUD7) Similar to protein kinase AtSIK (P0485B12.21 protein)	3.4e-114
SFR006.B05F990622	BE437659	homologue to UP|ALFC_ORYSA (Q40677) Fructose-bisphosphate aldolase chloroplast precursor (ALDP)	92.45	5.6e-63	TC205906	homologue to UP|ALFC_ORYSA (Q40677) Fructose-bisphosphate aldolase chloroplast precursor (ALDP)	3.1e-27
SFR006.B06F990623	BE437660	similar to UP|Q93Y95 (Q93Y95) Pollen signalling protein with adenylyl cyclase activity (Fragment)	77.5	8.1e-132	TC194190	similar to UP|Q93Y95 (Q93Y95) Pollen signalling protein with adenylyl cyclase activity (Fragment)	1.3e-29
SFR006.C01F990621	BE437666	homologue to UP|Q7XYC5 (Q7XYC5) Succinate dehydrogenase subunit 3 (Fragment)	91.47	1.7e-143	TC230848	UP|Q6ZH92 (Q6ZH92) Succinate dehydrogenase subunit 3	6.3e-66
SFR006.D01F990621	BE437678	homologue to UP|Q942X6 (Q942X6) Putative cytochrome c1	92.33	4.3e-162	TC202729	homologue to UP|Q942X6 (Q942X6) Putative cytochrome c1	6.0e-113
SFR006.D07F990622	BE437684	UP|ALEU_HORVU (P05167) Thiol protease aleurain precursor (EC 3.4.22.16)	100	3.9e-111	TC219521	homologue to UP|ALEU_HORVU (P05167) Thiol protease aleurain precursor (EC 3.4.22.16 EC 3.4.22.16)	1.7e-99
SFR006.D10F990623	BE437687	UP|VATL_AVESA (P23957) Vacuolar ATP synthase 16 kDa proteolipid subunit (EC 3.6.3.14 EC 3.6.3.6)	100	7.1e-96	TC219488	UP|VATL_AVESA (P23957) Vacuolar ATP synthase 16 kDa proteolipid subunit (EC 3.6.3.14 EC 3.6.3.14 EC 3.6.3.6)	4.3e-45
SFR006.D12F990705	BE437689	similar to UP|Q9ZT64 (Q9ZT64) Beta-glucosidase	75.26	4.6e-115	TC210399	weakly similar to UP|Q9ZT64 (Q9ZT64) Beta-glucosidase	8.6e-27
SFR006.F06F990625	BE437706	similar to UP|Q7F529 (Q7F529) Peroxiredoxin	87.04	5.3e-80	TC203417	similar to UP|Q8LLA7 (Q8LLA7) CI2C	4.0e-62
SFR006.G01F990621	BE437713	homologue to UP|Q6T6J8 (Q6T6J8) Salt-induced MAP kinase 1	92.35	1.9e-146	TC221301	UP|O81599 (O81599) MAP kinase homolog	1.9e-37
SFR006.G12F990705	BE437724	homologue to UP|O24342 (O24342) Serine/threonine kinase (EC 2.7.1.-)	95.45	3.1e-92	TC210731	homologue to UP|O24342 (O24342) Serine/threonine kinase (EC 2.7.1.-)	1.3e-62
SFR006.H02F990722	BE438175	homologue to UP|Q8RUU6 (Q8RUU6) Pyruvate decarboxylase	91.75	1.8e-134	TC188783	homologue to UP|Q8S4W8 (Q8S4W8) Pyruvate decarboxylase	3.7e-74
SFR007.A01F990616	BE437736	homologue to UP|Q6IWA4 (Q6IWA4) Cycloartenol synthase	93.75	5.7e-83	TC208296	homologue to UP|Q6IWA4 (Q6IWA4) Cycloartenol synthase	1.4e-59
SFR007.A08F990617	BE437743	homologue to UP|Q8LNY6 (Q8LNY6) G protein beta subunit	97.89	4.7e-122	TC213808	UP|Q8LNY6 (Q8LNY6) G protein beta subunit	1.1e-81
SFR007.B05F990618	BE437752	UP|Q7XYE8 (Q7XYE8) RUB1-conjugating enzyme (Fragment)	99.4	4.3e-45	TC204259	homologue to UP|Q7XYE8 (Q7XYE8) RUB1-conjugating enzyme (Fragment)	2.1e-91
SFR007.B08F990617	unknown	no hit			no hit	no hit	
SFR007.E06F990618	AF193803	homologue to UP|Q8H0K1 (Q8H0K1) Ethylene response element binding protein	97.7	9.3e-87	TC190926	UP|Q7XY26 (Q7XY26) EREBP transcription factor	3.1e-94
SFR007.G02F990625	BE437808	UP|O04875 (O04875) Phenylalanine ammonia-lyase (Fragment) (EC 4.3.1.5)	100	1.5e-53	TC205912	UP|PALY_WHEAT (Q43210) Phenylalanine ammonia-lyase (EC 4.3.1.5 EC 4.3.1.5 EC 4.3.1.5)	4.8e-54
SFR007.H10F990618	BE437827	similar to UP|Q6K551 (Q6K551) Putative serine/threonine protein kinase	74.51	5.7e-103	BM136273	similar to GP|27545044 putative protein kinase {Oryza sativa (japonica cultivar-group)}	1.6e-37
SFR008.A12F990510	BE437841	similar to UP|ARC1_LYCES (Q42884) Chorismate synthase 1 chloroplast precursor	76.58	1.8e-109	TC192248	similar to UP|ARC1_LYCES (Q42884) Chorismate synthase 1 chloroplast precursor	2.7e-94
SFR008.C12F990510	BE437865	UP|VAB2_HORVU (Q40079) Vacuolar ATP synthase subunit B isoform 2 (V-ATPase B subunit 2)	100	1.2e-85	TC190792	UP|VAB2_HORVU (Q40079) Vacuolar ATP synthase subunit B isoform 2 (V-ATPase B subunit 2)	6.7e-72
SFR008.D02F990625	BE437867	similar to GP|26338616 unnamed protein product {Mus musculus}	72.22	1.2e-42	CA646263	unknown	4.9e-07
SFR008.D11F990510	BE437876	similar to UP|SYFB_ARATH (Q9SGE9) Probable phenylalanyl-tRNA synthetase beta chain	74.17	1.2e-52	TC207597	similar to UP|SYFB_ARATH (Q9SGE9) Probable phenylalanyl-tRNA synthetase beta chain (Phenylalanine--tRNA ligase beta chain)	2.3e-38
SFR008.E08F990625	BE437885	similar to UP|Q6H7M1 (Q6H7M1) Putative fumarylacetoacetate hydrolase	89.98	5.6e-105	TC192853	similar to UP|Q6H7M1 (Q6H7M1) Putative fumarylacetoacetate hydrolase	5.7e-67

Gene ID	Gene accession	Putative function Barley database	% Identity	Barley database hit	Wheat database blast	Putative function Wheat database	Wheat database hit
SFR008.F02F990625	BE437891	UP|G3PX_HORVU (P26517) Glyceraldehyde-3-phosphate dehydrogenase cytosolic	100	2.3e-66	TC205817	UP|G3PX_HORVU (P26517) Glyceraldehyde-3-phosphate dehydrogenase cytosolic	2.4e-65
SFR008.F03F990625	BE437892	GP|11990901 ribulose-1 5-bisphosphate carboxylase/oxygenase small subunit {Triticum aestivum}	99.12	2.2e-80	TC219368	UP|Q9FRZ2 (Q9FRZ2) Ribulose-1 5-bisphosphate carboxylase/oxygenase small subunit	1.0e-61
SFR008.F06F990507	BE437895	PIR|T05920 probable cysteine proteinase - barley (fragment) {Hordeum vulgare;} (EC 3.4.22.-)	100	1.4e-92	TC205689	homologue to PIR|T05924 probable cysteine proteinase - barley (fragment) {Hordeum vulgare;} (EC 3.4.22.-)	1.7e-47
SFR008.F07F990625	BE437896	UP|Q94IC0 (Q94IC0) Betaine aldehyde dehydrogenase	100	2.8e-74	CD907718	GP|15147873 betaine aldehyde dehydrogenase {Hordeum vulgare subsp. vulgare}	7.8e-21
SFR008.F10F990628	BE437899	UP|G3PC_HORVU (P08477) Glyceraldehyde-3-phosphate dehydrogenase cytosolic (Fragment)	99.53	1.7e-115	TC205817	UP|G3PX_HORVU (P26517) Glyceraldehyde-3-phosphate dehydrogenase cytosolic	1.9e-64
SFR008.F10F990628	BE437899	UP|G3PC_HORVU (P08477) Glyceraldehyde-3-phosphate dehydrogenase cytosolic (Fragment)	99.53	1.7e-115	TC205817	UP|G3PX_HORVU (P26517) Glyceraldehyde-3-phosphate dehydrogenase cytosolic	1.9e-64
SFR008.G06F990628	BE437907	homologue to UP|Q7Y1F0 (Q7Y1F0) Putative glycine hydroxymethyltransferase	93.74	5.0e-155	TC218524	homologue to UP|Q7Y1F0 (Q7Y1F0) Putative glycine hydroxymethyltransferase	4.6e-150
SFR008.G11F990510	BE437912	homologue to UP|P93402 (P93402) Aspartate kinase-homoserine dehydrogenase precursor (EC 2.7.2.4 EC 1.1.1.3)	93.87	7.1e-163	BM137465	homologue to PIR|T03589 probable aspartate kinase (EC 2.7.2.4) / homoserine dehydrogenase (EC 1.1.1.3) precursor - rice	2.8e-63
SFR009.A04F990512	BE437927	UP|Q9ATV7 (Q9ATV7) Arabinoxylan arabinofuranohydrolase isoenzyme AXAH-II	100	1.9e-111	CA606633	GP|13398414 arabinoxylan arabinofuranohydrolase isoenzyme AXAH-II {Hordeum vulgare}	4.8e-62
SFR009.B06F990511	BE437941	similar to UP|KAD_PRUAR (O24464) Adenylate kinase (ATP-AMP transphosphorylase) (EC 2.7.4.3)	76.04	1.2e-105	TC226245	similar to UP|KAD_PRUAR (O24464) Adenylate kinase (ATP-AMP transphosphorylase) (EC 2.7.4.3 EC 2.7.4.3)	1.8e-91
SFR009.C02F990510	BE437948	similar to UP|Q9C7B3 (Q9C7B3) Protein phosphatase 2C putative;	75.73	3.0e-165	TC223740	similar to UP|Q9C7B3 (Q9C7B3) Protein phosphatase 2C putative	1.4e-133
SFR009.C09F990514	BE437955	similar to UP|O81606 (O81606) 1-aminocyclopropane-1-carboxylate oxidase (EC 1.4.3.-)	87.5	9.6e-63	TC188903	similar to UP|O81606 (O81606) 1-aminocyclopropane-1-carboxylate oxidase (EC 1.4.3.-)	3.0e-24
SFR009.C12F990511	unknown	no hit			no hit	no hit	
SFR009.D09F990628	BE437967	UP|JI23_HORVU (P32024) 23 kDa jasmonate-induced protein	100	7.0e-91	TC194718	similar to UP|JI23_HORVU (P32024) 23 kDa jasmonate-induced protein	1.0e-104
SFR009.E05F990511	BE437975	similar to UP|O23254 (O23254) Serine hydroxymethyltransferase (Serine methylase) (Glycine hydroxymethyltransferase)	84.58	1.9e-121	TC219189	homologue to UP|O23254 (O23254) Serine hydroxymethyltransferase (Serine methylase) (Glycine hydroxymethyltransferase)	6.1e-88
SFR009.E09F990628	BE437979	similar to UP|Q6YV24 (Q6YV24) Putative carbamoyl-phosphate synthetase small subunit	89.18	2.8e-99	TC204576	homologue to UP|Q6YV22 (Q6YV22) Carbamoyl-phosphate synthetase small subunit-like	1.5e-88
SFR009.F03F990512	BE437985	similar to UP|O82774 (O82774) Protein phosphatase 2A 55 kDa B regulatory subunit	89.72	1.3e-111	TC218836	homologue to UP|O82774 (O82774) Protein phosphatase 2A 55 kDa B regulatory subunit	4.9e-97
SFR009.G01F990510	BE437994	similar to UP|Q6K881 (Q6K881) Phosphatidylinositol 3-and 4-kinase-like	77.62	1.6e-164	TC220766	similar to UP|Q6K881 (Q6K881) Phosphatidylinositol 3-and 4-kinase-like	1.0e-150
SFR009.G03F990512	BE437996	UP|O24401 (O24401) Chlorophyll a/b-binding protein WCAB precursor	99.45	1.5e-57	TC221612	UP|O24401 (O24401) Chlorophyll a/b-binding protein WCAB precursor	2.4e-58
SFR009.G05F990511	BE437998	similar to PIR|T01617 probable protein kinase [imported] - Arabidopsis thaliana {Arabidopsis thaliana;}	72.22	5.3e-58	CK200635	weakly similar to PIR|T01617 probable protein kinase [imported] - Arabidopsis thaliana	3.0e-71
SFR009.H06F990511	AC006216	homologue to UP|Q9AV49 (Q9AV49) Putative homeodomain leucine zipper protein	92.1	8.7e-126	TC197089	homologue to UP|Q6TDS4 (Q6TDS4) Homeodomain leucine-zipper protein Hox9	7.5e-66
SFR009.H10F990514	BE438015	homologue to UP|Q84P58 (Q84P58) Adenosine kinase-like protein (Fragment)	94.38	1.1e-128	TC219234	homologue to UP|Q84P58 (Q84P58) Adenosine kinase-like protein (Fragment)	1.8e-123
SFR010.A05F990628	BE438022	homologue to UP|Q6ZA47 (Q6ZA47) Putative casein kinase 1 delta isoform 1	90.52	8.4e-110	TC188295	similar to UP|Q8L7Q2 (Q8L7Q2) Putative casein kinase	3.1e-99

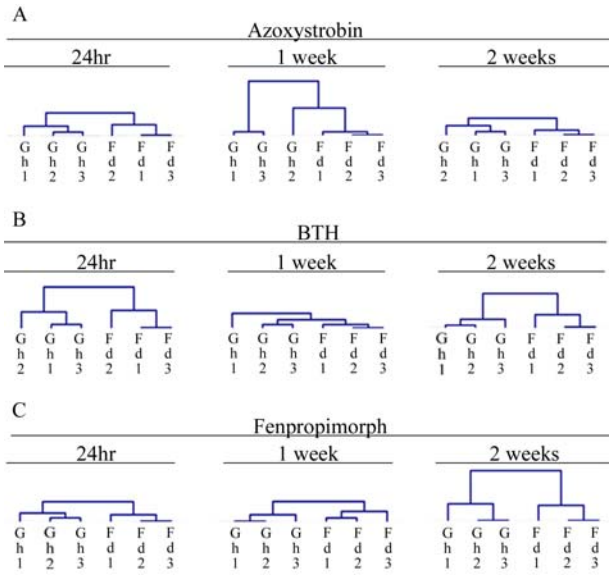
Gene ID	Gene accession	Putative function Barley database	% Identity	Barley database hit	Wheat database blast	Putative function Wheat database	Wheat database hit
SFR010.A09F990519	BE438026	homologue to UP|Q8H0P4 (Q8H0P4) NADP-dependant malate dehydrogenase (Fragment)	90.13	6.4e-86	TC203572	UP|MDHP_SORBI (P17606) Malate dehydrogenase [NADP] 1 chloroplast precursor (NADP-MDH-1)	3.9e-41
SFR010.A12F990603	BE438029	weakly similar to GP|14164492 putative protein kinase {Oryza sativa (japonica cultivar-group)}	69.23	7.6e-54	BQ483907	similar to GP|14164492 putative protein kinase {Oryza sativa (japonica cultivar-group)}	2.8e-47
SFR010.B08F990517	BE438037	similar to GP|13324784 putative protein kinase {Oryza sativa} [Oryza sativa (japonica cultivar-group)]	87.04	1.8e-68	TC196041	weakly similar to UP|Q93V58 (Q93V58) Putative serine threonine-protein kinase	8.1e-60
SFR010.B09F990519	BE438038	UP|TBP1_WHEAT (P26356) TATA-box binding protein 1 (TATA-box factor 1) (TATA binding factor 1)	100	6.6e-35	TC223082	UP|TBP1_WHEAT (P26356) TATA-box binding protein 1 (TATA-box factor 1) (TATA binding factor 1)	5.2e-34
SFR010.E06F990519	BE438069	similar to UP|Q8LLI2 (Q8LLI2) S-locus receptor-like kinase RLK11	78.24	1.0e-84	CD890867	similar to GP|22086620 S-locus receptor-like kinase RLK14 {Oryza sativa}	4.8e-40
SFR010.G09F990519	BE438096	homologue to UP|O24342 (O24342) Serine/threonine kinase (EC 2.7.1.-)	95.45	1.0e-86	TC210731	homologue to UP|O24342 (O24342) Serine/threonine kinase (EC 2.7.1.-)	1.2e-77
SFR010.G11F990722	BE438183	similar to UP|Q9XG98 (Q9XG98) Phosphoribosyl pyrophosphate synthase (EC 2.7.6.1)	79.58	6.1e-169	TC201477	similar to UP|Q9XG98 (Q9XG98) Phosphoribosyl pyrophosphate synthase (EC 2.7.6.1)	1.3e-140
SFR010.H02F990514	BE438100	similar to UP|Q40724 (Q40724) Transcriptional activator protein	79.21	9.7e-74	TC205037	similar to UP|Q40724 (Q40724) Transcriptional activator protein	1.5e-38
SFR010.H04F990517	BE438102	homologue to UP|Q84N52 (Q84N52) Phytoene synthase 2 (Fragment)	95.88	1.1e-62	CA643426	homologue to GP|18476085 phytoene synthase {Oryza sativa} [Oryza sativa (japonica cultivar-group)]	1.1e-25
SFR011.A09F990706	BE438115	homologue to UP|Q6UQ06 (Q6UQ06) Cytosolic glutathione reductase	97.93	1.3e-94	TC191149	UP|Q6UQ06 (Q6UQ06) Cytosolic glutathione reductase	2.5e-85
SFR011.B06F990706	BE438124	homologue to UP|Q8GTK9 (Q8GTK9) Putative asparaginyl-tRNA synthetase chloroplast/mitochondrial	91.08	2.6e-148	TC201849	homologue to UP|Q8GTK9 (Q8GTK9) Putative asparaginyl-tRNA synthetase chloroplast/mitochondrial	8.7e-94
SFR011.B12F990706	BE438129	homologue to UP|Q6H5W0 (Q6H5W0) Putative alcohol dehydrogenase	91.52	2.8e-140	TC218444	homologue to UP|ADHX_ORYSA (P93436) Alcohol dehydrogenase class III (Glutathione-dependent formaldehyde dehydrogenase) (FDH)	3.9e-24
SFR011.C01F990706	unknown	no hit			no hit	no hit	
SFR011.C12F990706	BE438140	homologue to UP|KADA_ORYSA (Q08479) Adenylate kinase A (ATP-AMP transphosphorylase) (EC 2.7.4.3)	91.29	5.3e-108	TC202876	homologue to UP|KADA_ORYSA (Q08479) Adenylate kinase A (ATP-AMP transphosphorylase) (EC 2.7.4.3 EC 2.7.4.3)	2.3e-97
SFR011.E01F990722	BE438191	homologue to UP|Q7XC38 (Q7XC38) Putative peptide methionine sulfoxide reductase	90.69	1.2e-74	TC192216	similar to UP|Q7XC38 (Q7XC38) Putative peptide methionine sulfoxide reductase	6.0e-60
wci1	TC132509	PIR|T04375 jacalin homolog - barley {Hordeum vulgare;}	99.67	2.0e-28	TAU32427	PIR|T06273 benzothiadiazole-induced protein (clone WCI-1) - wheat {Triticum aestivum;}	4.5e-265
wci2	TC139173	homologue to UP|Q42847 (Q42847) Lipoxygenase 2 (EC 1.13.11.12)	98.84	8.6e-138	TAU32428	UP|Q41520 (Q41520) Lipoxygenase (Fragment) (EC 1.13.11.12)	0.0
wci4	TC139286	PIR|T05920 probable cysteine proteinase - barley (fragment) {Hordeum vulgare;} (EC 3.4.22.-)	100	1.6e-72	TAU32430	homologue to UP|Q41522 (Q41522) Thiol protease	2.0e-295
wci5	TC139571	similar to PIR|T06278 benzothiadiazole-induced protein (clone WCI-5) - wheat {Triticum aestivum;}	89.91	9.4e-141	TAU32431	PIR|T06278 benzothiadiazole-induced protein (clone WCI-5) - wheat {Triticum aestivum;}	1.2e-189
wir1b	TC130810	homologue to UP|WIRA_WHEAT (Q01482) WIR1A protein	92.31	5.7e-29	WHTWIR1PR	UP|WIRB_WHEAT (Q01481) WIR1B protein	1.1e-102
wir1c	TC149589	homologue to UP|Q41581 (Q41581) WIR1 protein	90	6.5e-29	TARNAWIR1	UP|Q41581 (Q41581) WIR1 protein	3.5e-113
wir232	TC136076	UP|PR1C_HORVU (P32938) Pathogenesis-related protein 1C precursor	100	4.1e-122	TATHAU	UP|Q94F70 (Q94F70) Thaumatin-like protein	3.3e-129
wir5E123	TC146744	UP|Q8VWW3 (Q8VWW3) Glutathione transferase (EC 2.5.1.18)	100	1.2e-40	TAGSTA1	UP|GTH2_WHEAT (P30111) Glutathione S-transferase 2 (GST class-phi) (EC 2.5.1.18)	2.5e-121

Appendix 8.2

Pair-wise correlation coefficients of the different experimental treatments and time points shown in Figure 7. B: BTH treatment, F: Fenpropimorph treatment, A: Azoxystrobin treatment.

		B	F
24hr	F	0.66	
	A	0.02	0.21
1 week	F	0.52	
	A	0.87	0.62
2 weeks	F	0.38	
	A	0.41	0.73

Appendix 8.3



Cluster analysis of the whole microarray results after each treatment and each time point. Field and greenhouse results were analysed together. Fd1, 2 and 3: field samples; Gh 1, 2 and 3: greenhouse replicates. For most of the cases, a clear separation was observed between field and greenhouse results and good correlation was obtained between the replicates.

Appendix 8.4

Differentially expressed genes in field-grown non-treated plants compared to greenhouse-grown non-treated plants. Intensity ratios of genes determined to be differentially expressed by SAM analysis are in bold type. The positive values indicate higher gene expression in the field and negative values indicate higher gene expression in the greenhouse. The FDR were 4.5%, 3.2% and 5.5% for 24hr, one week and two weeks samples, respectively.

GeneID	Gene accession	Putative function	Mean 24hr	Mean 1 week	Mean 2 weeks
HV_CEb0010L20f	BE216529	UP|PR1_HORVU (Q05968) Pathogenesis-related protein 1	**9,8**	**5,9**	**7,2**
wir232	TATHAU	UP|Q94F70 (Q94F70) Thaumatin-like protein	**9,6**	**4,8**	**7,0**
HV_CEb0006J08f	BE215358	UP|PR1A_HORVU (P32937) Pathogenesis-related protein 1A/1B	**9,3**	**7,8**	**7,4**
wir1c	TARNAWIR1	UP|Q41581 (Q41581) WIR1 protein	**6,5**	**8,7**	**12,0**
HV_CEb0009I06f	BE216122	similar to UP|Q9XEN6 (Q9XEN6) Chitinase IV	**6,3**	**4,9**	**6,1**
HV_CEb0003A01f	BE214283	UP|Q43764 (Q43764) Chitinase (EC 3.2.1.14)	**5,7**	**6,5**	**5,3**
gluc2	TC225609	UP|Q9XEN5 (Q9XEN5) Beta-1 3-glucanase	**5,5**	**4,4**	**5,5**
HV_CEb0024H14f	BE559397	UP|PR12_HORVU (P35792) Pathogenesis-related protein PRB1-2	**4,5**	**3,8**	2,3
HV_CEb0010G19f	BE216411	UP|E13B_HORVU (P15737) Glucan endo-1 3-beta-glucosidase GII precursor	**4,2**	**3,0**	3,0
HV_CEb0009D03f	BE216036	similar to UP|Q9XEN6 (Q9XEN6) Chitinase IV	**3,6**	**2,9**	**3,0**
SFR001.D01F990616	BE437324	UP|Q8L3P8 (Q8L3P8) Stem rust resistance protein Rpg1	**3,5**	**3,2**	1,7
HV_CEb0003B05f[a]	BE214500	homologue to UP|WIRA_WHEAT (Q01482) WIR1A protein	3,4	**6,6**	6,0
pox381	X56011	UP|Q43212 (Q43212) Peroxidase precursor (EC 1.11.1.7)	**3,1**	**6,0**	**24,9**
wci5	TAU32431	PIR|T06278 Benzothiadiazole-induced protein clone WCI-5 wheat	**3,1**	1,7	**4,4**
HV_CEb0002C16f	BE214080	UP|ENPL_HORVU (P36183) Endoplasmin homolog precursor (GRP94 homolog)	**3,0**	1,2	2,0
HV_CEb0024H02f	BE559387	UP|Q43764 (Q43764) Chitinase (EC 3.2.1.14)	**3,0**	**5,6**	**4,8**
HV_CEb0003K12f	BE214507	UP|P93180 (P93180) Pathogenesis-related protein 4	3,0	**4,4**	**4,1**
HV_CEb0009B05f	BE216003	homologue to UP|Q9FRT5 (Q9FRT5) Monosaccharide transporter 3	2,6	**3,3**	1,4
HV_CEb0021P01f	BE519980	UP|Q40068 (Q40068) Peroxidase (EC 1.11.1.7)	**2,5**	**5,2**	**4,1**
SFR006.D07F990622	BE437684	UP|ALEU_HORVU (P05167) Thiol protease aleurain precursor (EC 3.4.22.16)	**2,2**	2,4	1,6
HV_CEb0016N18f	BE519542	UP|Q9SME4 (Q9SME4) Glutathione peroxidase-like protein	**2,2**	**2,4**	1,7
HVSMEh0088J22f	BE195219	UP|ALA2_HORVU (P52894) Alanine aminotransferase 2	**2,0**	1,2	1,9
HV_CEb0003J11f	BE214483	UP|Q43765 (Q43765) Chitinase (EC 3.2.1.14)	2,0	**3,5**	1,9
HV_CEb0003P20f	BE214619	UP|CHS1_HORVU (P26018) Chalcone synthase 1	1,6	**10,8**	**3,7**
HV_CEb0004G09f	TC140105	similar to UP|Q8GT52 (Q8GT52) Hexose transporter	1,5	**2,9**	1,1
HVSMEg0006B08f[a]	BG343672	homologue to GB|BAD18000 Serine/threonine protein kinase SAPK4 {*Oryza sativa* }	1,5	**2,8**	0,8
HVSMEg0001A02f	BE230858	homologue to UP|Q84N28 (Q84N28) Caffeic acid O-methyltransferase	1,4	**2,7**	**5,0**
HV_CEb0006A03f	BE215152	UP|O04876 (O04876) Phenylalanine ammonia-lyase (Fragment) (EC 4.3.1.5)	1,4	**2,5**	1,6
HVSMEg0003O15f	AW982677	UP|CHS1_HORVU (P26018) Chalcone synthase 1	1,4	**11,7**	**4,1**
HV_CEb0007F10f[a]	BI955817	homologue to UP|Q9AXS1 (Q9AXS1) Putative thiamine biosythesis protein ThiC	1,3	**2,8**	0,5
wir5E123	TAGSTA1	UP|GTH2_WHEAT (P30111) Glutathione S-transferase 2 (EC 2.5.1.18)	1,2	**2,9**	1,5
HVSMEg0016D20f	BG344820	homologue to UP|Q6I573 (Q6I573) Putative 1-deoxy-D-xylulose-5-phosphate synthase	0,4	**3,5**	1,3
HV_CEb0011B08f	BE216646	similar to GP|19849279 Cyt-P450 monooxygenase [*Oryza sativa*]	-2,3	**-3,3**	-1,0
HVSMEg0006G05f	BG343558	UP|INO1_HORVU (O65195) Inositol-3-phosphate synthase	**-2,9**	-1,4	-1,5
HV_CEa0016M13f	BF267043	UP|Q9M6N6 (Q9M6N6) RNase S-like protein	**-8,3**	**-10,4**	**-5,7**

[a]: Clones that have not given the same sequencing result as the Clemson University.

Appendix 8.5

False discovery rate percentages determined between 24 hrs and two weeks after treatment with either 2,4-D, cinidon-ethyl (Ci), and tribenuron-methyl (Tr) in controlled conditions and field trials.

	Controlled			Field		
	2,4-D	**Ci**	**Tr**	**2,4-D**	**Ci**	**Tr**
24 hrs	3	4	23	ng	5	30
72 hrs	32	ng	20	18	10	7
1 week	19	11	11	6	4	ng
2 weeks	23	20	31	20	100	43

ng: no gene determined as differentially expressed

Appendix 8.6

Differentially expressed genes in the trial in controlled conditions 72 hrs after treatment with either 2,4-D, cinidon-ethyl (C), and tribenuron-methyl (T). Intensity ratios of genes determined to be differentially expressed by SAM analysis are in bold type. The positive values indicate gene induction and negative values indicate gene repression.

Gene ID	Accession number	Putative function	2,4-D	C	T
pox381	TC140187	UP|Q40068 (Q40068) Peroxidase (EC 1.11.1.7)	**1.5**	1.1	1.1
HV_CEb0003K23f	BE214516	UP|Q7XJ26 (Q7XJ26) Iron/ascorbate-dependent oxidoreductase	**1.5**	1.1	1.1
HVSMEg0013J22f	BE060858	homologue to UP|ZB14_MAIZE (P42856) 14 kDa zinc-binding protein (Protein kinase C inhibitor) (PKCI)	**1.3**	1.1	1.2
HV_CEb0004M23f	BG299484	UP|Q7XTK5 (Q7XTK5) IAA1 protein	1.2	1.1	**1.7**
HVSMEg0002O09f	AW982323	homologue to UP|Q9LKM0 (Q9LKM0) Nucleoside diphosphate kinase (EC 2.7.4.6)	1.2	1.0	**1.5**
HVSMEg0002O22f[a]	BE231062	homologue to UP|Q9LKM0 (Q9LKM0) Nucleoside diphosphate kinase (EC 2.7.4.6)	1.2	1.1	**1.4**
HVSMEg0003N10f	BI951269	similar to GP|21553536 receptor-like protein kinase {Arabidopsis thaliana}	1.0	-1.2	**1.6**
HVSMEh0080A22f[a]	AAA34292	homologue to UP|Q6WV72 (Q6WV72) Histone H4	-1.1	1.3	**2.0**

[a] : clones that have not given the same blast result as in the database.

Appendix 8.7

Differentially expressed genes in the trial in controlled conditions two weeks after treatment with either 2,4-D, cinidon-ethyl (C), and tribenuron-methyl (T). Intensity ratios of genes determined to be differentially expressed by SAM analysis are in bold type. The positive values indicate gene induction and negative values indicate gene repression.

Gene ID	Accession number	Putative function	2,4-D	C	T
HV_CEb0017L05f	BE558442	weakly similar to UP|Q84NG8 (Q84NG8) Putative receptor kinase	-2.2	1.3	**-1.0**
HV_CEb0003P20f	BE214619	UP|CHS1_HORVU (P26018) Chalcone synthase 1	-2.1	-1.5	**-1.8**
HV_CEb0015B10f	BE558194	weakly similar to GP|22535653 putative protein kinase Xa21 receptor type precursor {Oryza sativa (japonica cultivar-group)}	-1.8	1.1	**-1.1**
HVSMEg0003O15f	AW982677	UP|CHS1_HORVU (P26018) Chalcone synthase 1	-1.7	-1.5	**-1.4**
HV_CEb0021J19f	BE519892	similar to UP|Q8H8H7 (Q8H8H7) Putative flavanone 3-hydroxylase	**-1.7**	-1.0	-1.5
HVSMEg0016D20f	BG344820	homologue to UP|Q6I573 (Q6I573) Putative 1-deoxy-D-xylulose-5-phosphate synthase	**-1.6**	-1.4	-2.1
HV_CEb0008F10f	unknown	unknown	-1.6	**-1.4**	-1.2
HV_CEb0005E11f	unknown	unknown	-1.3	**-1.3**	-1.2
SFR008.D02F990625	BE437867	similar to GP|26338616 unnamed protein product {Mus musculus}	-1.2	**-1.4**	-1.6
HVSMEg0015D01f	BE455799	UP|Q9MAY8 (Q9MAY8) Endo-1 4-beta-glucanase Cel1	-1.2	**-1.4**	-1.3
HVSMEg0012E23f[a]	BG343757	similar to UP|Q8RZH3 (Q8RZH3) Putative CTP synthase	**-1.2**	-1.6	-2.4
HVSMEg0001P11f	BF261118	similar to UP|Q9FYP0 (Q9FYP0) Putative peroxidase	-1.1	**-1.5**	-1.3

[a] : clones that have not given the same blast result as in the database.

Appendix 8.8

Differentially expressed genes in the field trial two weeks after treatment with either 2,4-D, cinidon-ethyl (C), and tribenuron-methyl (T). Intensity ratios of genes determined to be differentially expressed by SAM analysis are in bold type. The positive values indicate gene induction and negative values indicate gene repression.

Gene ID	Accession number	Putative function	2,4-D	C	T
HV_CEb0010E08f	BE216352	imilar to UP|Q7XHB3 (Q7XHB3) Putative peroxidase	1.1	-1.4	**-1.6**
HVSMEg0002G13f	AW982232	JP|Q9MAY8 (Q9MAY8) Endo-1 4-beta-glucanase Cel1	-1.0	**-1.8**	-1.4
HV_CEb0011B08f	BE216646	imilar to GP|19849279 Cyt-P450 monooxygenase {Oryza sativa} [Oryza sativa japonica cultivar-group)]	**-1.4**	-1.2	-1.3
HVSMEg0006D08f	BG343678	omologue to UP|Q9S711 (Q9S711) ESTs C22657(S0014) (Transmembrane protein kinase)	-1.6	-1.4	**-1.7**
HV_CEb0018K12f	AV836098	omologue to UP|Q9LD61 (Q9LD61) Aspartate carbamoyl transferase	**-1.6**	-1.1	-1.4
SFR003.H05F990621	BE437451	GP|11990901 ribulose-1 5-bisphosphate carboxylase/oxygenase small subunit {Triticum aestivum}	**-1.7**	1.0	1.4
SFR009.G03F990512	BE437996	JP|O24401 (O24401) Chlorophyll a/b-binding protein WCAB precursor	**-1.9**	-1.1	-1.0
SFR004.A07F990621	BE437465	imilar to UP|Q75I52 (Q75I52) Expressed protein	**-2.1**	1.0	-1.3

8. 9 Acknowledgements

I would like to thank Beat Keller for giving me the opportunity to do my thesis in his laboratory. I am grateful for his support during these laborious years and for his constructive critics during the writing.

I especially want to express my gratitude to Edwige Isidore, Nabila Yahiaoui and Catherine Feuillet, my good fairies, for their constant support and their joyful help that were necessary to overcome the bad moments during all these years here…

I also would like to thank Röbi Dudler (Tonton Röbi) for his helpful comments and the good times during the Praktikum.

I am grateful to Nils Stein and Philippe Reymond for their help during the setting up of the capricious microarray technique. The musketeers from the Functionnal Genomic Center Zürich: Jens Sobeck, Andrea Patrignani and Ulrich Wagner have also been a considerable source of advice in order to find the best spot (Jens), to prepare the cRNA samples (Andrea) and to analyse the data (Uli). Many thanks to the librarian who found "old" or inaccessible papers and to Hanspeter Schmidhauser and Daniel Bollier who participated to the improvement of the arrayer device.

My special thanks go to Sophie, Payorm and Paolo who, together with Nabila and Edwige, created a really great atmosphere in and out the P3-12 lab.

Many thanks to all lab members (past and present) from all the groups (Keller, Dudler and Grossniklaus) for their advice, help and friendship. I will hardly forget Roshani, Célia, Anouck, Silvia, Kathrin, Bea, Livia, Eligio, Simon and Barbara (vielen Dank für die Zusammenfassung!), Raja, Ratan, Olivier, Philippa, José, Vladimir, Umut, Jay, Juan, Amal and all the members of the Institutes of the Botanischen Garten (as well as the firemen of Zürich!).

Merci à Papa et Maman, Charlotte, Jean-Luc et Colin, à Dany, Thomas et Julien pour leur éternel et inconditionnel soutien.

8.10 Currivulum vitae

May 21, 1976	Born in Dinard (Ille-et-Vilaine), France
1990-1993	High School in Saint-Malo, France
June 1993	Baccalauréat in Science (D) with distinction in Saint-Malo, France
1994-1999	Master of Science in Plant Biology with distinction at the University of Rennes, France
June 2001	PhD at the Institute of Plant Biology, University of Zürich